

09/388090

FILE 'CAPLUS' ENTERED AT 09:43:38 ON 22 MAR 2001

L4 1688 SEA FILE=CAPLUS ABB=ON PLU=ON NEISSERIA(S) (POLYPEPTIDE
OR PEPTIDE OR PROTEIN OR POLYPROTEIN)
L5 609 SEA FILE=CAPLUS ABB=ON PLU=ON L4 AND (DNA OR DEOXYRIBON
CULEIC OR DEOXY RIBONUCLEIC OR NUCLEOTIDE)
L6 19 SEA FILE=CAPLUS ABB=ON PLU=ON L5 AND SDS(W) (PAGE OR
(POLYACRYL? OR POLY ACRYL?) (1W)ELECTROPHOR?)

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L6 ANSWER 1 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:569562 CAPLUS

DOCUMENT NUMBER: 133:292765

TITLE: Two glycosylase/abasic lyases from Neisseria
mucosa that initiate DNA repair at
sites of UV-induced photoproducts

AUTHOR(S): Nyaga, Simon G.; Lloyd, R. Stephen

CORPORATE SOURCE: Center for Molecular Science, The University of
Texas Medical Branch, Galveston, TX, 77555, USA

SOURCE: J. Biol. Chem. (2000), 275(31), 23569-23576

CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular
Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Diverse organisms ranging from Escherichia coli to humans contain a
variety of DNA repair proteins that function in the
removal of damage caused by shortwave UV light. This study reports
the identification, purifn., and biochem. characterization of two
DNA glycosylases with assocd. abasic lyase activity from
Neisseria mucosa. These enzymes, pyrimidine dimer glycosylase I and
II (Nmu-pdg I and Nmu-pdg II), were purified 30,000- and
10,000-fold, resp. SDS-poly-acrylamide
gel electrophoresis anal. indicated that Nmu-pdg I is
approx. 30 kDa, whereas Nmu-pdg II is approx. 19 kDa. The
N-terminal amino acid sequence of Nmu-pdg II exhibits 64 and 66%
identity with E. coli and Hemophilus parainfluenzae endonuclease
III, resp. Both Nmu-pdg I and Nmu-pdg II were found to have broad
substrate specificities, as evidenced by their ability to incise
DNA contg. many types of UV and some types of oxidative
damage. Consistent with other glycosylase/abasic lyases, the
existence of a covalent enzyme-DNA complex could be
demonstrated for both Nmu-pdg I and II when reactions were carried
out in the presence of sodium borohydride. These data indicate the
involvement of an amino group in the catalytic reaction mechanism of
both enzymes.

REFERENCE COUNT: 33

REFERENCE(S): (2) Bailly, V; Biochemistry 1987, V242, P565

CAPLUS

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- (3) Bailly, V; Biochemistry 1989, V259, P751
CAPLUS
- (4) Cunningham, R; Ann N Y Acad Sci 1994, V726,
P215 CAPLUS
- (5) Dizdaroglu, M; Mutat Res 1996, V362, P1
CAPLUS
- (6) Dodson, M; Biochemistry 1993, V32, P8284
CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:277031 CAPLUS

DOCUMENT NUMBER: 133:172879

TITLE: Genetic characterization of pilin glycosylation
in *Neisseria meningitidis*

AUTHOR(S): Power, Peter M.; Roddam, Louise F.; Dieckelmann,
Manuela; Srikhanta, Yogitha N.; Tan, Yoke Cheng;
Berrington, Andrew W.; Jennings, Michael P.

CORPORATE SOURCE: Department of Microbiology and Parasitology, The
University of Queensland, Brisbane, 4072,
Australia

SOURCE: Microbiology (Reading, U. K.) (2000), 146(4),
967-979

CODEN: MROBEO; ISSN: 1350-0872

PUBLISHER: Society for General Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Pili of *Neisseria meningitidis* are a key virulence factor, being the major adhesin of this capsulate organism and contributing to specificity for the human host. Pili are post-translationally modified by addn. of an O-linked trisaccharide, Gal(.beta.1-4)Gal(.alpha.1-3)2,4-diacetimidido-2,4,6-trideoxyhexose. In a previous study the authors identified and characterized a gene, *pglA*, encoding a galactosyltransferase involved in pilin glycosylation. In this study a set of random genomic sequences from *N. meningitidis* strain MC58 was used to search for further genes involved in pilin glycosylation. Initially, an open reading frame was identified, and designated *pglD* (pilin glycosylation gene D), which was homologous to genes involved in polysaccharide biosynthesis. The region adjacent to this gene was cloned and **nucleotide** sequence anal. revealed two further genes, *pglB* and *pglC*, which were also homologous with genes involved in polysaccharide biosynthesis. Insertional mutations were constructed in *pglB*, *pglC* and *pglD* in *N. meningitidis* C311#3, a strain with well-defined LPS and pilin-linked glycan structures, to det. whether these genes had a role in the biosynthesis of either of these mols. Anal. of these mutants revealed that there was no alteration in the phenotype of LPS in any of the mutant strains as judged by **SDS-PAGE** gel migration. In contrast, increased

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gel migration of the pilin subunit mols. of pglB, pglC and pglD mutants by Western anal. was obsd. Pilin from each of the pglB, pglC and pglD mutants did not react with a terminal-galactose-specific stain, confirming that the gel migration differences were due to the alteration or absence of the pilin-linked trisaccharide structure in these mutants. In addn., antisera specific for the C311#3 trisaccharide failed to react with pilin from the pglB, pglC, pglD and galE mutants. Anal. of nucleotide sequence homologies has suggested specific roles for pglB, pglC and pglD in the biosynthesis of the 2,4-diacetimido-2,4,6-trideoxyhexose structure.

REFERENCE COUNT: 60

REFERENCE(S): (2) Allen, A; Mol Microbiol 1996, V19, P37
CAPLUS
(3) Altschul, S; Nucleic Acids Res 1997, V25, P3389 CAPLUS
(4) Annunziato, P; J Bacteriol 1995, V177, P312 CAPLUS
(6) Awram, P; J Bacteriol 1998, V180, P3062 CAPLUS
(7) Bechthold, A; Mol Gen Genet 1995, V248, P610 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:53852 CAPLUS

DOCUMENT NUMBER: 132:106952

TITLE: Screening of Neisseria vaccine candidates and vaccines against pathogenic neisseria

INVENTOR(S): Ala'aldeen, Dlawer; Todd, Ian

PATENT ASSIGNEE(S): The University of Nottingham, UK

SOURCE: PCT Int. Appl., 51 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000003003	A2	20000120	WO 1999-GB2205	19990709
WO 2000003003	A3	20000413		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, Searcher : Shears 308-4994

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DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9947917 A1 20000201 AU 1999-47917 19990709
PRIORITY APPLN. INFO.: GB 1998-14902 19980710
 WO 1999-GB2205 19990709

AB Methods of screening for vaccine candidates, vaccines against pathogenic neisseria and intermediaries for such vaccines have been developed. Two vaccine candidates TspA and TspB have been identified and characterized which either alone or in conjunction with the vaccines provide for treatment against pathogenic neisserias in particular Neisseria meningitidis and/or Neisseria gonorrhoeae.

L6 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:682300 CAPLUS

DOCUMENT NUMBER: 132:32985

TITLE: Neisseria gonorrhoeae bacterioferritin:
 structural heterogeneity, involvement in iron
 storage and protection against oxidative stress

AUTHOR(S): Chen, Cheng-Yen; Morse, Stephen A.

CORPORATE SOURCE: Division of AIDS, Sexually Transmitted Diseases
 and Tuberculosis Laboratory Research, Centers
 for Disease Control and Prevention, National
 Centers for Infectious Disease, Atlanta, GA,
 30333, USA

SOURCE: Microbiology (Reading, U. K.) (1999), 145(10),
 2967-2975

CODEN: MROBEO; ISSN: 1350-0872

PUBLISHER: Society for General Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The iron-storage protein bacterioferritin (Bfr) from *Neisseria gonorrhoeae* strain F62 was identified in cell-free exts. and subsequently purified by column chromatog. Gonococcal Bfr had an estd. mol. mass of 400 kDa by gel filtration; however, anal. by SDS-PAGE revealed that it was composed of 18 kDa (BfrA) and 22 kDa (BfrB) subunits. DNA encoding BfrB was amplified by PCR using degenerate primers derived from the N-terminal amino acid sequence of BfrB and from a C-terminal amino acid sequence of *Escherichia coli* Bfr. The DNA sequence of bfrA was subsequently obtained by genome walking using single-specific-primer PCR. The two Bfr genes were located in tandem with an intervening gap of 27 bp. A potential Fur-binding sequence (12 of 19 bp identical to the consensus neisserial fur sequence) was located within the 5' flanking region of bfrA in front of a putative -35 hexamer. The homol. between the DNA sequences of bfrA and bfrB was 55.7%; the deduced amino acid sequences of BfrA (154 residues) and BfrB (157 residues) showed 39.7% identity, and showed 41.3% and 56.1% identity, resp., to E.

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coli Bfr. Expression of recombinant BfrA and BfrB in *E. coli* strain DH5.alpha. was detected on Western blots probed with polyclonal anti-*E. coli* Bfr antiserum. Most Bfrs are homopolymers with identical subunits; however, the evidence presented here suggests that gonococcal Bfr was composed of two similar but not identical subunits, both of which appear to be required for the formation of a functional Bfr. A Bfr-deficient mutant was constructed by inserting the .OMEGA. fragment into the BfrB gene. The growth of the BfrB-deficient mutant in complex medium was reduced under iron-limited conditions. The BfrB-deficient mutant was also more sensitive to killing by H₂O₂ and paraquat than the isogenic parent strain. These results demonstrate that gonococcal Bfr plays an important role in iron storage and protection from iron-mediated oxidative stress.

REFERENCE COUNT: 46
 REFERENCE(S): (1) Andrews, S; Eur J Biochem 1993, V213, P329
 CAPLUS
 (2) Andrews, S; FEBS Lett 1991, V293, P164
 CAPLUS
 (3) Andrews, S; J Bacteriol 1989, V171, P3940
 CAPLUS
 (4) Andrews, S; J Inorg Biochem 1992, V47, P161
 CAPLUS
 (5) Braun, V; Biol Chem 1997, V378, P779 CAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:624824 CAPLUS
 DOCUMENT NUMBER: 127:315311
 TITLE: The AroQ and PheA domains of the bifunctional
 P-protein from *Xanthomonas campestris* in a
 context of genomic comparison
 AUTHOR(S): Gu, Wei; Williams, Donna S.; Aldrich, Henry C.;
 Xie, Gary; Gabriel, Dean W.; Jensen, Roy A.
 CORPORATE SOURCE: Department of Microbiology and Cell Science,
 University of Florida, Gainesville, FL, USA
 SOURCE: Microb. Comp. Genomics (1997), 2(2), 141-158
 CODEN: MCGEFP; ISSN: 1090-6584
 PUBLISHER: Liebert
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The gene (denoted aroQp.cntdot.pheA) encoding the bifunctional
 P-protein (chorismate mutase-P/prephenate dehydratase) from
Xanthomonas campestris was cloned. AroQp.cntdot.pheA is essential
 for L-phenylalanine biosynthesis. DNA sequencing of the
 smallest subclone capable of functional complementation of an
Escherichia coli phenylalanine auxotroph revealed a putative open
 reading frame (ORF) of 1200 bp that would encode a 43,438-Da
 protein. AroQp.cntdot.PheA exhibited 51% amino acid identity with a
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Pseudomonas stutzeri homolog and greater than 30% identities with AroQp.cntdot.PheA proteins from *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and a no. of enteric bacteria. AroQp.cntdot.PheA from *X. campestris*, when expressed in *E. coli*, possesses a 40-residue amino-terminal extension that is lysine-rich and that is absent in all of the AroQp.cntdot.PheA homologues known at present. About 95% of AroQp.cntdot.PheA was particulate and readily sedimented by low-speed centrifugation. Sol. preps. of cloned AroQp.cntdot.PheA exhibited a native mol. mass of 81,000 Da, indicating that the active enzyme species is a homodimer. These preps. were unstable after purifn. of about 40-fold, even in the presence of glycerol, which was an effective protectant before fractionation. When AroQp.cntdot.PheA was overproduced by a T7 translation vector, unusual inclusion bodies having a macromol. structure consisting of protein fibrils were obsd. by electron microscopy. Insol. protein collected at low-speed centrifugation possessed high catalytic activity. The single band obtained via SDS-PAGE was used to confirm the translational start via N-terminal amino acid sequencing. A perspective on the evolutionary relationships of monofunctional AroQ and PheA proteins and the AroQp.cntdot.PheA family of proteins is presented. A *serC* gene located immediately upstream of *X. campestris* aroQp.cntdot.pheA appears to reflect a conserved gene organization, and both may belong to a single transcriptional unit.

L6 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:43957 CAPLUS

DOCUMENT NUMBER: 126:100085

TITLE: Analysis of the *icsBA* locus required for biosynthesis of the inner core region from *Neisseria meningitidis* lipopolysaccharide

AUTHOR(S): van der Ley, Peter; Kramer, Marco; Martin, Adele; Richards, James C.; Poolman, Jan T.

CORPORATE SOURCE: Laboratory of Vaccine Development and Immune Mechanisms, National Institute of Public Health and the Environment, Bilthoven, 3720 BA, Neth.

SOURCE: FEMS Microbiol. Lett. (1997), 146(2), 247-253
CODEN: FMLED7; ISSN: 0378-1097

PUBLISHER: Elsevier

DOCUMENT TYPE: Journal

LANGUAGE: English

AB By deletion mutagenesis in the entire meningococcal chromosome, we have previously identified the *icsA* gene, which encodes the glycosyltransferase required for adding GlcNAc to Hep-II in the inner core of meningococcal LPS. This gene has homol. to several LPS glycosyltransferases, notably to *rfaK* from *Salmonella typhimurium* and *bplH* from *Bordetella pertussis*, both of which encode GlcNAc transferases. Directly upstream of *icsA* is an ORF showing significant homol. to the hypothetical protein HI0653 from the

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Haemophilus influenzae genome sequence, and to a lesser degree to putative glycosyltransferases from *Streptococcus thermophilus* and *Yersinia enterocolitica*. Insertional inactivation of this ORF resulted in a meningococcal strain with truncated LPS. We have named this new LPS-involved gene *icsB*. Differences in binding of monoclonal antibodies and in mobility on Tricine-SDS-PAGE showed that LPS from *icsA* and *icsB* mutants is similar but not identical. On the basis of these results, we postulated that the new gene encodes the glycosyltransferase required for adding Glc to Hep-I. Structural anal. of purified mutant LPS by electrospray mass spectrometry was used to verify this hypothesis. The compn. detd. for *icsA* and *icsB* is lipid-A-(KDO)2-(Hep)2.PEA and lipid-A-(KDO)2-(Hep)2.PEA-GlcNAc, resp. The *icsA* and *icsB* genes thus form an operon encoding the glycosyltransferases required for chain elongation from the lipid-A-(KDO)2-(Hep)2 basal structure, with *IcsA* first adding GlcNAc to Hep-II and *IcsB* subsequently adding Glc to Hep-I. Only then is completion of the lacto-N-neotetraose structure possible through the action of the *lgtA-E* genes.

L6 ANSWER 7 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:460738 CAPLUS
 DOCUMENT NUMBER: 125:134350
 TITLE: Cloning, complementation, and characterization of an *rfaE* homolog from *Neisseria gonorrhoeae*
 AUTHOR(S): Levin, James C.; Stein, Daniel C.
 CORPORATE SOURCE: Department Microbiology, University Maryland, College Park, MD, 20742, USA
 SOURCE: J. Bacteriol. (1996), 178(15), 4571-4575
 CODEN: JOBAAY; ISSN: 0021-9193
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB *Neisseria gonorrhoeae* WS1 is a spontaneous pyocin (a bacteriocin produced by *Pseudomonas aeruginosa*)-resistant mutant of *N. gonorrhoeae* FA19 that produces a truncated lipooligosaccharide (LOS) and is nontransformable. The LOS-specific mutation in WS1 was moved into a transformable background by transforming FA19 with chromosomal DNA from WS1 (generating strain JWS-1). A clone (pJCL2) capable of restoring JWS-1 to wild-type LOS expression, as detected by its acquisition of reactivity with monoclonal antibodies and by its complemented SDS-PAGE profile, was isolated. Sequential unidirectional deletion and DNA sequence anal. of pJCL2 identified an open reading frame, designated *lsi-7*, that could complement the defect in JWS-1. Homol. searches against various databases indicated that *lsi-7* had homol. with several *Escherichia coli* genes involved in the phosphorylation of sugars. *Lsi-7* is adjacent to the *lsi-6* gene, another gene involved in LOS biosynthesis. Complementation studies using *Salmonella typhimurium* lipopolysaccharide mutants showed *lsi-6* and *lsi-7* to be gonococcal

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homologs of *S. typhimurium* rfaD and rfaE, resp. Reverse transcriptase PCR anal. demonstrated that lsi-6 and lsi-7 are part of the same transcriptional unit.

L6 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:176101 CAPLUS

DOCUMENT NUMBER: 124:252269

TITLE: Identification of a locus involved in meningococcal lipopolysaccharide biosynthesis by deletion mutagenesis

AUTHOR(S): van der Ley, Pete; Kramer, Marco; Steeghs, Liana; Kuipers, Betsy; Andersen, Svein R.; Jennings, Michael P.; Moxon, E. Richard; Poolman, Jan T.

CORPORATE SOURCE: Lab. Vaccine Development and Immune Mechanisms, Natl. Inst. Public Health and Environmental Protection, Bilthoven, 3720 BA, Neth.

SOURCE: Mol. Microbiol. (1996), 19(5), 1117-25
CODEN: MOMIEE; ISSN: 0950-382X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A novel method for insertion/deletion mutagenesis in meningococci was devised. This consisted of ligating a digest of total chromosomal DNA to a 1.1 kb restriction fragment contg. an erythromycin-resistance marker (ermC), and subsequent transformation of the ligation mixt. into the homologous meningococcal strain H44/76. Southern blotting of a no. of the resulting erythromycin-resistant transformants demonstrated that all carried the ermC gene inserted at different positions in the chromosome. Mutants with a specific phenotype were identified by screening with the anti-lipopolysaccharide (LPS) monoclonal antibody MN4A8B2, which is specific for immunotype L3. In this way, two independent L3-neg. mutant strains were isolated. In transformation expts. with chromosomal DNA from these mutants, erythromycin-resistance and lack of MN4A8B2 reactivity were always linked, showing that the insertion/deletion was in a locus involved in LPS biosynthesis. On SDS-PAGE, the mutant LPS displayed an electrophoretic mobility intermediate between that produced by the previously isolated galeE and rfaF mutant strains. Chem. anal. of the mutant LPS revealed that the structure was probably lipid A-(KDO)2-(Hep)2. Chromosomal DNA flanking the ermC insertion in these two mutant strains was cloned, and used as probe for the isolation of the corresponding region of the wild-type strain. From hybridization and polymerase chain reaction (PCR) anal., it could be concluded that both mutations map to the same locus. The affected gene probably encodes the glycosyltransferase necessary for adding N-acetylglucosamine to heptose.

L6 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:93922 CAPLUS
 DOCUMENT NUMBER: 124:167034
 TITLE: Identification and characterization of genes required for post-translational modification of *Campylobacter coli* VC167 flagellin
 AUTHOR(S): Guerry, Patricia; Doig, Peter; Alm, Richard A.; Burr, Donald H.; Kinsella, Niamh; Trust, Trevor J.
 CORPORATE SOURCE: Enteric Diseases Program, Naval Medical Res. Inst., Bethesda, MD, 20889, USA
 SOURCE: Mol. Microbiol. (1996), 19(2), 369-78
 CODEN: MOMIEE; ISSN: 0950-382X
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Two genes have been identified in *Campylobacter coli* VC167 which are required for the biosynthesis of posttranslational modifications on flagellin proteins. The *ptmA* gene encodes a protein of predicted Mr 28486 which shows significant homol. to a family of alc. dehydrogenases from a variety of bacteria. The *ptmB* gene encodes a **protein** of predicted Mr 26598 with significant homol. to CMP-N-acetylneuraminic acid synthetase enzymes involved in sialic acid capsular biosynthesis in *Neisseria meningitidis* and *Escherichia coli* K1. Site-specific mutation of either *ptmA* or *ptmB* caused loss of reactivity with antisera specific to the post-translational modifications and a change in the isoelec. focusing fingerprints relative to the parent strains. Mutation of *ptmB*, but not of *ptmA*, caused a change in apparent Mr of the flagellin subunit in **SDS-PAGE** gels. The *ptmA* and *ptmB* genes are present in other strains of *Campylobacter*. In a rabbit model the *ptmA* mutant showed a reduced ability to elicit protection against subsequent challenge with heterologous strains of the same Lior serotype compared to the parental wild-type strain. This suggests that the surface-exposed post-translational modifications may play a significant role in the protective immune response.

L6 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:5055 CAPLUS
 DOCUMENT NUMBER: 124:108167
 TITLE: Molecular analysis of a locus for the biosynthesis and phase-variable expression of the lacto-N-neotetraose terminal lipopolysaccharide structure in *Neisseria meningitidis*
 AUTHOR(S): Jennings, Michael P.; Hood, Derek, W.; Peak, Ian R. A.; Virji, Mumtaz; Moxon, E. Richard
 CORPORATE SOURCE: Molecular Infectious Diseases Group and Department of Paediatrics, Institute of Searcher : Shears 308-4994

SOURCE: Molecular Medicine, Headington, OX3 9DU, UK
Mol. Microbiol. (1995), 18(4), 729-40
CODEN: MOMIEE; ISSN: 0950-382X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Lipopolysaccharide (LPS) is a major determinant of *Neisseria meningitidis* virulence. A key feature of meningococcal LPS is the phase-variable expression of terminal structures which are proposed to have disparate roles in pathogenesis. In order to identify the biosynthetic genes for terminal LPS structures and the control mechanisms for their phase-variable expression, the *lic2A* gene, which is involved in LPS biosynthesis in *Haemophilus influenzae*, was used as a hybridization probe to identify a homologous gene in *N. meningitidis* strain MC58. The homologous region of DNA was cloned and nucleotide sequence anal. revealed three open reading frames (ORFs), two of which were homologous to the *H. influenzae lic2A* gene. All three ORFs were mutagenized by the insertion of antibiotic-resistance cassettes and the LPS from these mutant strains was analyzed to det. if the genes had a role in LPS biosynthesis. Immunol. and tricine-SDS-PAGE anal. of LPS from the mutant strains indicated that all three genes were probably transferases in the biosynthesis of the terminal lacto-N-neotetraose structure of meningococcal LPS. The first ORF of the locus contains a homopolymeric tract of 14 guanosine residues within the 5'-end of the coding sequence. As the lacto-N-neotetraose structure in meningococcal LPS is subject to phase-variable expression, colonies that no longer expressed the terminal structure, as detd. by monoclonal antibody binding, were isolated. Anal. of an "off" phase variant revealed a change in the no. of guanosine residues resulting in a frameshift mutation, indicating that slipped-strand mispairing mechanism, operating in the first ORF, controls the phase-variable expression of lacto-N-neotetraose.

L6 ANSWER 11 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:545656 CAPLUS

DOCUMENT NUMBER: 123:193240

TITLE: A mutation in the *Neisseria gonorrhoeae rfaD* homolog results in altered lipo-oligosaccharide expression

AUTHOR(S): Drazek, E. Susan; Stein, Daniel C.; Deal, Carolyn D.

CORPORATE SOURCE: Dep. Bacterial Diseases, Walter Reed Army Inst. Res., Washington, DC, 20307-5100, USA

SOURCE: J. Bacteriol. (1995), 177(9), 2321-7
CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The gonococcal *lsi-6* locus was cloned and shown by DNA Searcher : Shears 308-4994

sequence anal. to have homol. with the *E. coli* rfaD gene, which encodes ADP-L-glycero-D-mannoheptose epimerase. This enzyme is involved in the biosynthesis of the lipopolysaccharide precursor ADP-L-glycero-D-mannoheptose. A site-directed frameshift mutation in *lsi-6* was constructed by PCR amplification and introduced into the chromosome of *Neisseria gonorrhoeae* MS11 P+ by transformation. The lipo-oligosaccharides (LOS) of mutant and parental strains were characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (**SDS-PAGE**). The *lsi-6* mutant produced LOS components with apparent mol. masses of 2.6 and 3.6 kDa as compared with a 3.6-kDa band of the MS11 P+ strain. The parental LOS phenotype was expressed when a revertant was constructed by transformation of the cloned wild-type gene into the *lsi-6* mutant. The immunoreactivity of LOS from parental and constructed strains was examd. by **SDS-PAGE** and Western blotting. Only the parental and reconstructed wild-type strains produced a 3.6-kDa LOS component that reacted with monoclonal antibody 2-1-L8. These results suggest that the *lsi-6* locus is involved in gonococcal LOS biosynthesis and that the nonreactive mutant 3.6-kDa LOS component contains a conformational change or altered saccharide compn. that interferes with immunoreactivity.

L6 ANSWER 12 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:292406 CAPLUS

DOCUMENT NUMBER: 122:124946

TITLE: Lipooligosaccharide biosynthesis in *Neisseria gonorrhoeae*: Cloning, identification and characterization of the .alpha.1,5 heptosyltransferase I gene (rfaC)

AUTHOR(S): Zhou, Daoguo; Lee, Na-Gyong; Apicella, Michael A.

CORPORATE SOURCE: Department Microbiology, University Iowa, Iowa City, IA, 52242, USA

SOURCE: Mol. Microbiol. (1994), 14(4), 609-18
CODEN: MOMIEE; ISSN: 0950-382X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The identical partial deep-core structure of Hep.alpha.1-3Hep.alpha.1-5KDO in *Salmonella typhimurium* LT2 LPS and *Neisseria gonorrhoeae* LOS enabled us to isolate a DNA fragment from *N. gonorrhoeae* that was able to complement the .alpha.1,5 LOS heptosyltransferase defect in the *S. typhimurium* rfaC630 (SA1377) mutant. **SDS-PAGE** anal. confirmed the prodn. of wild-type LPS in the transformant. Subcloning revealed that complementation was due to a 1.2 kb fragment. Sequence anal. revealed a complete open reading frame capable of encoding a 36-37 kDa peptide. In vitro transcription-translation anal. of the 1.2 kb clone confirmed that a 37 kDa protein was encoded by this DNA fragment. The DNA sequence-deduced protein

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had 36% identity and 58% similarity to *S. typhimurium* heptosyltransferase I (RfaC). Primer extension anal. indicated that transcription of the cloned gene in *N. gonorrhoeae* strain 1291 begins 144 bp upstream of the start codon at a G nucleotide. An isogenic mutant of *N. gonorrhoeae* strain 1291 with an m-Tn3 insertion inside the coding sequence expressed a single truncated LOS with a similar mol. mass to *S. typhimurium* rfaC LPS. We conclude that the 1.2 kb fragment encodes the .alpha.1,5 LOS heptosyltransferase I (RfaC) in *N. gonorrhoeae*. Our studies also provide further evidence that the third KDO residue in *S. typhimurium* LPS is added after the core synthesis is completed.

L6 ANSWER 13 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:107608 CAPLUS

DOCUMENT NUMBER: 122:234202

TITLE: Genetic locus for the biosynthesis of the variable portion of *Neisseria gonorrhoeae* lipooligosaccharide

AUTHOR(S): Gotschlich, Emil C.

CORPORATE SOURCE: Lab. Bacterial Pathogenesis Immunol., Rockefeller Univ., New York, NY, 10021-6399, USA

SOURCE: J. Exp. Med. (1994), 180(6), 2181-90

CODEN: JEMEAV; ISSN: 0022-1007

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A locus involved in the biosynthesis of gonococcal lipooligosaccharide (LOS) has been cloned from gonococcal strain F62. The locus contain five open reading frames. The first and second reading frames are homologous, but not identical, to the fourth and fifth reading frames, resp. Interposed is an addnl. reading frame which has distant homol. to the *Escherchia coli* rfaI and rfaJ genes, both glucosyl transferases involved in lipopolysaccharide core biosynthesis. The second and fifth reading frames show strong homol. to the lex-1 of lic2A gene of *Haemophilus influenzae*, but do not contain the CAAT repeats found in this gene. Deletions of each of these five genes, of combinations of genes, and of the entire locus were constructed and introduced into parental gonococcal strain F62 by transformation. The LOS phenotypes were then analyzed by SDS-PAGE and reactivity with monoclonal antibodies. Anal. of the gonococcal mutants indicates that four of these genes are the glycosyl transferases that add GalNAc.beta.1 .fwdarw. 3Gal.beta.1 .fwdarw. 4GalNAc.beta.1 .fwdarw. 3Gal.beta.1 .fwdarw. 4 to the substrate Glc.beta.1 .fwdarw. 4Hep .fwdarw. R of the inner core region. The gene with homol. to *E. coli* rfaI/rfaJ is involved with the addn. of the .alpha.-linked galactose residue in the biosynthesis of the alternative LOS structure Gal.alpha.1 .fwdarw. Gal.beta.1 .fwdarw. 4Gal.beta.1 .fwdarw. 4Hep .fwdarw. R. Since these genes encode LOS glycosyl transferases they have been named lgtA, lgtB, lgtC, lgtD, and lgtE.

Searcher : Shears 308-4994

The DNA sequence anal. revealed that lgtA, lgtC, and lgtD contained poly-G tracts, which, in strain F62 were, resp., 17, 10, and 11 bp. Thus, three of the LOS biosynthetic enzymes are potentially susceptible to premature termination by reading frame changes. It is likely that these structural features are responsible for the high-frequency genetic variation of gonococcal LOS.

L6 ANSWER 14 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:404768 CAPLUS

DOCUMENT NUMBER: 121:4768

TITLE: Lipooligosaccharide biosynthesis in pathogenic *Neisseria*. Cloning, identification, and characterization of the phosphoglucomutase gene

AUTHOR(S): Zhou, Daoguo; Stephens, David S.; Gibson, Bradford W.; Engstrom, Jeffrey J.; McAllister, Carl F.; Lee, Frank K. N.; Apicella, Michael A.

CORPORATE SOURCE: Dep. Microbiol., Univ. Iowa, Iowa City, IA, 52242, USA

SOURCE: J. Biol. Chem. (1994), 269(15), 11162-9
CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The lipooligosaccharide (LOS) of pathogenic *Neisseria* is an important factor in disease pathogenesis. Little is known about the genes involved in neisserial LOS biosynthesis. To elucidate specific LOS biosynthetic genes, the authors screened a Tn916 library that was constructed in *Neisseria meningitidis* strain NMB. This strain expresses a single LOS that has an mol. mass of 4.5 kDa and binds monoclonal antibody (mAb) 3F11. This library was screened using a mAb panel that recognizes structural differences in neisserial LOS oligosaccharides. A stable LOS mutant of strain NMB was identified which the authors designated NMB-R6. This mutant expressed an LOS with an mol. mass of approx. 3.1-3.2 kDa and did not bind mAb 3F11. Genomic DNA from this mutant transformed *N. meningitidis* strain NMB to the tetracycline resistant NMB-R6 phenotype greater than 10⁻⁴/recipient/.mu.g of DNA. In addn., the authors transformed *Neisseria gonorrhoeae* strain 1291 (LOS phenotype mol. mass 4.5 kDa, mAb 3F11+) to the NMB-R6 LOS phenotype with *N. meningitidis* NMB-R6 genomic DNA. Anal. of *N. gonorrhoeae* strain 1291-R6 LOS by mass spectroscopy showed that the LOS oligosaccharide structure is GlcNAc .fwdarw. Hep2phosphoethanolamine .fwdarw. 2-keto-3-deoxymannooctulosonic acid (where Hep is heptose). Sequence anal. showed that the transposon is inserted into the 3' end of a gene that has homol. to the human phosphoglucomutase (PGM) gene. Sequence comparison indicated that the putative PGM gene in *N. gonorrhoeae* 1291 and *N. meningitidis* NMB had 92% identity at the DNA level. PGM and glucokinase activity was present in cell free exts. of *N. meningitidis* NMB and

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N. gonorrhoeae strain 1291. N. meningitidis NMB-R6 and N. gonorrhoeae strain 1291-R6 had no detectable PGM activity, whereas glucokinase activity was similar to the wild type strains. PGM activity can be reconstituted in N. meningitidis strain NMB-R6 by transformation with the cloned PGM gene. **SDS-polyacrylamide gel electrophoresis** demonstrated that NMB-R6 transformed with the PGM gene expressed the 3F11+, 4.5-kDa LOS of the parent NMB strain. The inability of N. meningitidis NMB-R6 and N. gonorrhoeae strain 1291-R6 to convert glucose 6-phosphate to glucose 1-phosphate results in the truncated LOS phenotype expressed by these mutants.

L6 ANSWER 15 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:212128 CAPLUS

DOCUMENT NUMBER: 120:212128

TITLE: Pilus-facilitated adherence of Neisseria meningitidis to human epithelial and endothelial cells: modulation of adherence phenotype occurs concurrently with changes in primary amino acid sequence and the glycosylation status of pilin

AUTHOR(S): Virji, Mumtaz; Saunders, Jon R.; Sims, Gail; Makepeace, Katherine; Maskell, Duncan; Ferguson, David J. P.

CORPORATE SOURCE: Dep. Paediatr., Univ. Oxford, Oxford, OX3 9DU, UK

SOURCE: Mol. Microbiol. (1993), 10(5), 1013-28

CODEN: MOMIEE; ISSN: 0950-382X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Adherence of capsulate N. meningitidis to endothelial and epithelial cells is facilitated in variants that express pili. Whereas piliated variants of N. meningitidis strain C311 adhered to endothelial cells in large nos. (>150 bacteria/cell), derivs. contg. specific mutations that disrupt pile encoding the pilin subunit were both non-piliated and failed to adhere to endothelial cells (<1 bacterium/cell). In addn., meningococcal pili recognized human endothelial and epithelial cells but not cells originating from other animals. Variants of strain C311 were obtained that expressed pilins of reduced apparent Mr and exhibited a marked increase in adherence to epithelial cells. Structural anal. of pilins from 2 hyper-adherent variants and the parent strain were carried out by DNA sequencing of their pile genes. Deduced mol. wts. of pilins were considerably lower than their apparent Mr values on **SDS-PAGE**. Hyperadherent pilins shared unique changes in sequence, including substitution of Asn-113 for Asp-113 and changes from Asn-Asp-Thr-Asp to Thr-Asp-Ala-Lys at residues 127-130 in mature pilin. Asn residues 113 and 127 of parental pilin both form part of the typical eukaryotic N-glycosylation motif Asn-X-Ser/Thr and could potentially be glycosylated

Searcher : Shears 308-4994

post-translationally. The presence of carbohydrate on pilin was demonstrated and when pilins were deglycosylated, their migration on **SDS-PAGE** increased, supporting the notion that variable glycosylation accounts for discrepancies in apparent and deduced mol. wts. Functionally distinct pilins produced by 2 fully piliated variants of a 2nd strain (MC58) differed only in that the putative glycosylation motif Asn-60-Asn-61-Thr-62 in an adherent variant was replaced with Asp-60-Asn-61-Ser-62 in a nonadherent variant. Fully adherent backswitchers obtained from the nonadherent variant always regained Asn-60 but retained Ser-62. It is proposed, therefore, that functional variations in *N. meningitidis* pili may be modulated in large part by primary amino acid sequence changes that ablate or create N-linked glycosylation sites on the pilin subunit.

L6 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:28863 CAPLUS

DOCUMENT NUMBER: 120:28863

TITLE: Expression of meningococcal epitopes in LamB of *Escherichia coli* and the stimulation of serosubtype-specific antibody responses

AUTHOR(S): McCarvil, J.; McKenna, A. J.; Grief, C.; Hoy, C. S.; Sesardic, D.; Maiden, M. C. J.; Feavers, I. M.

CORPORATE SOURCE: Div. Bacteriol., Natl. Inst. Biol. Stand. and Control, South Mimms/Potters Bar, EN6 3QG, UK

SOURCE: Mol. Microbiol. (1993), 10(1), 203-13

CODEN: MOMIEE; ISSN: 0950-382X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The class 1 outer membrane protein (OMP), a major variable surface antigen of *Neisseria meningitidis*, is a component of novel meningococcal vaccines currently in field trials. Serol. variants of the protein are also used to serosubtype meningococci. Most of the amino acid changes that give rise to antigenic variants of the protein occur in 2 variable regions (VR1 and VR2) that are thought to form loops on the cell surface. The polymerase chain reaction. (PCR) was used to amplify the nucleotide sequences encoding VR1 and VR2 from the chromosomal DNA of *N. meningitidis* strain M1080. These were cloned in frame into the LamB gene of the *Escherichia coli* expression vector pAJC264. Whole-cell ELISAs, using monoclonal antibodies, and **SDS-PAGE** confirmed that, upon induction, strains of *E. coli* carrying these constructs expressed hybrid LamB proteins contg. the *N. meningitidis* surface loops. These strains were used to immunize rabbits and the resultant polyclonal antisera reacted specifically with the class I OMP of ref. strain M1080 (P1.7). Immunogold labeling of meningococcal cells and whole-cell dot-blot analyses with these antisera showed that the variable epitopes were exposed on the cell surface and confirmed that this approach could be used

Searcher : Shears 308-4994

to obtain serosubtype-specific antisera. The binding profiles of the antisera were detd. from their reactions with overlapping synthetic peptides and their reactivity compared with that of relevant serosubtype-specific monoclonal antibodies. This approach was used successfully to raise antisera against 2 other class 1 OMP VR2s. A fourth antiserum raised against a VR2, including the P1.1 epitope, was not subtype specific.

L6 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1993:226653 CAPLUS

DOCUMENT NUMBER: 118:226653

TITLE: Identification of meningococcal serosubtypes by polymerase chain reaction

AUTHOR(S): Maiden, Martin C. J.; Bygraves, Jane A.; McCarvil, James; Feavers, Ian M.

CORPORATE SOURCE: Nal. Inst. Biol. Stand. Control, Hertsfordshire, EN6 3QG, UK

SOURCE: J. Clin. Microbiol. (1992), 30(11), 2835-41
CODEN: JCMIDW; ISSN: 0095-1137

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The polymerase chain reaction was used as the basis of a novel typing method for *Neisseria meningitidis*. Southern hybridization expts. demonstrated that it was possible to identify genes encoding different serol. variants of the meningococcal class 1 outer membrane protein by probing with polymerase chain reaction products corresponding to known epitopes. A set of 14 defined variable regions was prepd. in bacteriophage M13mp19 by the cloning of polymerase chain reaction products. The phage were dot blotted onto membrane filters, which were used as targets for hybridization of radiolabeled amplified class 1 outer membrane protein genes. Thus, the presence of many different subtype-specific epitopes could be investigated in one expt. This technique was evaluated with a set of serol. ref. strains, mainly of serogroup B organisms, and provided an alternative, rapid, and comprehensive typing system that was capable of distinguishing known serosubtypes and also of defining currently untypeable strains independently of SDS -polyacrylamide gel electrophoresis or serol. anal. An addnl. advantage of this technique was that in the case of an unknown serosubtype (i.e., one that did not hybridize with any of the known samples), the DNA amplified from the original sample could be used to det. the nucleotide sequence of the novel serosubtype and to clone the corresponding variable region into bacteriophage M13. It may be possible to develop this procedure for the diagnostic detection and typing of meningococci directly from clin. samples even when culture is not possible because of antibiotic treatment of an acute case.

L6 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2001 ACS

Searcher : Shears 308-4994

09/7388090

ACCESSION NUMBER: 1990:546610 CAPLUS
DOCUMENT NUMBER: 113:146610
TITLE: Stable expression of meningococcal class 1
protein in an antigenically reactive form in
outer membranes of Escherichia coli
AUTHOR(S): White, D. A.; Barlow, A. K.; Clarke, I. N.;
Heckels, J. E.
CORPORATE SOURCE: Med. Sch., Univ. Southampton, Southampton, SO9
4XT, UK
SOURCE: Mol. Microbiol. (1990), 4(5), 769-76
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The entire gene encoding the class 1 outer membrane protein of *Neisseria meningitidis* is located on a 2.2 kb fragment obtained on digestion of chromosomal DNA with XbaI. This XbaI fragment from strain MC50 (subtype P1-16), which had previously been cloned in bacteriophage M13, was transferred to the plasmid vector pMTL20. The resulting plasmid (pPORA100) was propagated in *E. coli* (JM109) and cell lysates were subjected to SDS-PAGE. Western blotting with anti-class I protein antibodies revealed constitutive expression of a protein of 41 kD, corresponding to the class 1 protein of the parent meningococcal strain, which was absent in the *E. coli* control. Fractionation of *E. coli* cells carrying the recombinant plasmid revealed that the protein exclusively located in the outer membrane, and N-terminal amino acid anal. of the expressed protein revealed that normal processing of the signal peptide had occurred. Immunogold electron microscopy showed that the protective epitope recognized by a P1-16 subtype-specific monoclonal antibody was exposed in an antigenically reactive form on the surface of *E. coli* cells carrying plasmid pPORA100. In contrast, expression in *E. coli* of a 2nd plasmid (pPORA 104) lacking the coding sequence for the 1st 15 amino acids of the signal peptide resulted in accumulation of recombinant class 1 protein only in the cytoplasm of the cells. Thus, the presence of the meningococcal signal sequence ensures expression of this meningococcal porin protein in an antigenically native conformation in outer membrane of *E. coli*, while its absence results in expression of a sol. protein. Such constructs illustrate the potential use of recombinant DNA technol. for the development of effective human vaccines against meningococcal infection.

L6 ANSWER 19 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1982:488519 CAPLUS
DOCUMENT NUMBER: 97:88519
TITLE: Genetic linkage between serogroup specificity
and antibiotic resistance in *Neisseria*
gonorrhoeae
Searcher : Shears 308-4994

09/7388090

AUTHOR(S): Bygdeman, Solgun; Baeckman, Marianne;
Danielsson, Dan; Norgren, Mari
CORPORATE SOURCE: Dep. Clin. Bacteriol., Karolinska Inst.,
Stockholm, Swed.
SOURCE: Acta Pathol., Microbiol. Immunol. Scand., Sect.
B (1982), 90B(3), 243-50
CODEN: APMMD2
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Significant differences were demonstrated in antibiotic susceptibility between gonococcal strains of the recently described W serogroups, W I, W II, and W III. Strains of serogroup W I were almost always sensitive to penicillin and other antibiotics, whereas those of the W II and W III serogroups showed a higher incidence of decreased susceptibility. Transformation expts. were undertaken with an antibiotic sensitive serogroup W I gonococcal strain as recipient and a multiresistant W II strain as DNA-donor. Transformants with increased resistance to penicillin and several other antibiotics as compared with the recipient acquired the same serogroup specificity as the W II donor. With one of these W II transformants as donor and the sensitive W I strain as recipient, all transformants acquired the same antibiotic susceptibility pattern and serogroup as the donor. **SDS-polyacrylamide gel electrophoresis**, performed on Sarkosyl-extd. outer membrane proteins from donor, recipient, and some transformants, showed an increase in the mol. wt. of the Protein I of the outer membrane of the W II transformants as compared with that of the recipient strain. In rocket-line and crossed-line immunoelectrophoresis the W II transformants could not be distinguished from the W II donor strain. A genetic linkage between antibiotic multiresistance and serogroup W II specificity was thus shown. This is in agreement with the demonstrated higher incidence of W II strains with increased antibiotic resistance as compared with that of serogroup W I strains.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 09:57:13 ON 22 MAR 2001)

L7 48 S L6
L8 24 DUP REM L7 (24 DUPLICATES REMOVED)

L8 ANSWER 1 OF 24 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2001:86340 BIOSIS
DOCUMENT NUMBER: PREV200100086340
TITLE: Analysis of lipooligosaccharide biosynthesis in the
Neisseriaceae.
AUTHOR(S): Arking, Dan; Tong, Yanhong; Stein, Daniel C. (1)
CORPORATE SOURCE: (1) Department of Cell Biology and Molecular
Genetics, University of Maryland, College Park, MD,
20742: DS64@UMAIL.UMD.EDU USA
Searcher : Shears 308-4994

SOURCE: Journal of Bacteriology, (February, 2001) Vol. 183,
No. 3, pp. 934-941. print.
ISSN: 0021-9193.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Neisserial lipooligosaccharide (LOS) contains three oligosaccharide chains, termed the alpha, beta, and gamma chains. We used Southern hybridization experiments on DNA isolated from various *Neisseria* spp. to determine if strains considered to be nonpathogenic possessed DNA sequences homologous with genes involved in the biosynthesis of these oligosaccharide chains. The presence or absence of specific genes was compared to the LOS profiles expressed by each strain, as characterized by their mobilities on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and their reactivities with various LOS-specific monoclonal antibodies. A great deal of heterogeneity was seen with respect to the presence of genes encoding glycosyltransferases in *Neisseria*. All pathogenic species were found to possess DNA sequences homologous with the lgt gene cluster, a group of genes needed for the synthesis of the alpha chain. Some of these genes were also found to be present in strains considered to be nonpathogenic, such as *Neisseria lactamica*, *N. subflava*, and *N. sicca*. Some nonpathogenic *Neisseria* spp. were able to express high-molecular-mass LOS structures, even though they lacked the DNA sequences homologous with rfaF, a gene whose product must act before gonococcal and meningococcal LOS can be elongated. Using a PCR amplification strategy, in combination with DNA sequencing, we demonstrated that *N. subflava* 44 possessed lgtA, lgtB, and lgtE genes. The predicted amino acid sequence encoded by each of these genes suggested that they encoded functional proteins; however, structural analysis of LOS isolated from this strain indicated that the bulk of its LOS was not modified by these gene products. This suggests the existence of an additional regulatory mechanism that is responsible for the limited expression of these genes in this strain.

L8 ANSWER 2 OF 24 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

ACCESSION NUMBER: 2000-293149 [25] WPIDS

DOC. NO. CPI: C2000-088672

TITLE: Isolated outer membrane protein from a *Moraxella catarrhalis* strain used for diagnosis treatment and prevention of disease caused by *M. catarrhalis* e.g. pneumonia, otitis media and respiratory infections.

DERWENT CLASS: B04 D16

INVENTOR(S): TILLMANN, U F; TUCKER, K

PATENT ASSIGNEE(S): (ANTE-N) ANTEX BIOLOGICS INC

COUNTRY COUNT: 87

PATENT INFORMATION:

Searcher : Shears 308-4994

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000018910	A1	20000406	(200025)*	EN	108
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC					
MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES					
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK					
LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG					
SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9964100	A	20000417	(200035)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000018910	A1	WO 1999-US22918	19991001
AU 9964100	A	AU 1999-64100	19991001

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9964100	A Based on	WO 200018910

PRIORITY APPLN. INFO: US 1998-164714 19981001

AN 2000-293149 [25] WPIDS

AB WO 200018910 A UPAB: 20000524

NOVELTY - An isolated or substantially purified outer membrane protein (OMP), OMP21, from a *Moraxella catarrhalis* strain with an apparent molecular weight of 16-20 kD as determined by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a peptide fragment of OMP21 which specifically binds to an antibody that specifically binds OMP21;
- (2) an isolated nucleic acid molecule (I) encoding OMP21, a complementary sequence, a sequence substantially homologous to, or any fragment of OMP21;
- (3) plasmid pOMP21X obtainable from *Escherichia coli* Top10F' (pOMP21X) deposited as ATCC 98878;
- (4) a recombinant expression vector (II) adapted for transformation of a host cell comprising (I) or the plasmid of (3);
- (5) a recombinant expression vector (III) adapted for transformation of a host cell comprising (I) and an expression system operatively coupled to a nucleic acid molecule for expression by the host of OMP21;
- (6) a transformed host cell containing (II) or (III);

Searcher .: Shears 308-4994

(7) an isolated recombinant OMP21 producible by the transformed host cell of (6);

(8) an attenuated or inactivated cultivar of *M. catarrhalis* where the cultivar has been genetically manipulated to delete the nucleic acid encoding OMP21 so it is non-transcribed;

(9) a pharmaceutical composition which is prophylactic, therapeutic, or immunogenic including a vaccine, or a vaccine comprising at least one component selected from:

(a) OMP21;

(b) (I);

(c) OMP21, obtained from a transformed host comprising an expression vector containing (I) and a means of expression coupled to the nucleic acid for expression of OMP21 by the host;

(d) a recombinant vector comprising (I); and

(e) a transformed cell comprising the vector of (d);

(10) antisera raised against the compositions or vaccine of (9);

(11) an isolated antibody (IV) present in the antisera of (10) that specifically binds one or more of the components present in the compositions or vaccine of (9);

(12) a method for detecting anti-*M. catarrhalis* antibodies in a test sample comprising:

(a) contacting a test sample with the composition of (9) in the presence of anti-*M. catarrhalis* antibodies to form antigen:anti-*M. catarrhalis* antibody immunocomplexes; and

(b) detecting any immunocomplexes formed as an indication of the presence of anti-*M. catarrhalis* antibodies in the test sample;

(13) a diagnostic kit for detecting antibodies to *M. catarrhalis* comprising the pharmaceutical compositions of (9), a container and a reagent for detecting *M. catarrhalis* antigen:anti-*M. catarrhalis* antibody immunocomplexes formed between the compositions and the sample;

(14) a method for detecting the presence of *M. catarrhalis* in a test sample comprising contacting a test sample with the antibodies of (11) and detecting any immunocomplexes formed as an indication of the presence of *M. catarrhalis* in the test sample;

(15) a diagnostic kit for detecting the presence of *M. catarrhalis* comprising the antibodies of (11), a container and a reagent for measuring *M. catarrhalis*:anti-*M. catarrhalis* antibody immunocomplexes formed between the antibodies and *M. catarrhalis*;

(16) a method for determining the presence of nucleic acid encoding OMP21 in a sample comprising contacting a sample with (I) to produce duplexes comprising (I) and any nucleic acid molecule encoding OMP21 in the sample specifically hybridizable with (I) and detecting the duplexes produced;

(17) a diagnostic kit for determining the presence of nucleic acid encoding OMP21 in a sample comprising (I), a device for contacting (I) with a test sample and a device for detecting duplexes produced; and

(18) a method of preventing, treating or ameliorating a disorder related to *M. catarrhalis* in an animal in need of treatment comprising administering an effective amount of the compositions or vaccine of (9).

ACTIVITY - Antibacterial; auditory; antiinflammatory.

MECHANISM OF ACTION - Vaccine.

Pre-immune serum and anti-OMP21 antiserum was examined for activity in mediating complement killing of *M. catarrhalis* using the Serum Bactericidal Test described by Zollinger et al in Immune responses to *Neisseria meningitis*, Manual of Clinical Laboratory Immunology, 3rd ed., 347-349 with cells of *M. catarrhalis* strains not *N. meningitis* cells. The anti-OMP21 antiserum mediated complement-killing of *M. catarrhalis* ATCC 49143 but not of a deletion mutant of *M. catarrhalis* with the OMP21 gene disrupted.

USE - OMP21, its nucleic acids and antibodies can be used in prophylactic and therapeutic compositions for treating a *M. catarrhalis* bacterial infection, otitis media, respiratory infections, sinusitis and pneumonia (claimed). They are useful as reagents for the clinical or medical diagnosis of *M. catarrhalis* infections and for scientific research on the properties of pathogenicity, virulence and infectivity of *M. catarrhalis* and host defense mechanisms.

The antibodies, particularly those that are cytotoxic may be used in passive immunization to prevent or attenuate *M. catarrhalis* infections of animals e.g. humans.

Dwg.0/9

L8 ANSWER 3 OF 24 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD.

ACCESSION NUMBER: 2000-256581 [22] WPIDS

CROSS REFERENCE: 2000-237782 [20]

DOC. NO. CPI: C2000-078252

TITLE: *Neisseria meningitidis* NMA SP
polypeptide, nucleotide sequences
and antibodies, useful in vaccines against
infection.

DERWENT CLASS: B04 D16

INVENTOR(S): HARRIS, A M; JACKSON, W J

PATENT ASSIGNEE(S): (ANTE-N) ANTEX BIOLOGICS INC

COUNTRY COUNT: 85

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
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WO 2000012535	A2	20000309	(200022)*	EN	75
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RW:	AT	BE	CH	CY	DE	DK	EA	ES	FI	FR	GB	GH	GM	GR	IE	IT	KE	LS	LU	MC
MW	NL	OA	PT	SD	SE	SL	SZ	UG	ZW											

W:	AE	AL	AM	AT	AU	AZ	BA	BB	BG	BR	BY	CA	CH	CN	CU	CZ	DE	DK	EE	ES
	FI	GB	GD	GE	GH	GM	HR	HU	ID	IL	IN	IS	JP	KE	KG	KP	KR	KZ	LC	LK
	LR	LS	LT	LU	LV	MD	MG	MK	MN	MW	MX	NO	NZ	PL	PT	RO	RU	SD	SE	SG

Searcher : Shears 308-4994

09/7388090

SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW
AU 9957894 A 20000321 (200031)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000012535	A2	WO 1999-US19663	19990901
AU 9957894	A	AU 1999-57894	19990901

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9957894	A Based on	WO 200012535

PRIORITY APPLN. INFO: US 1998-98685 19980901

AN 2000-256581 [22] WPIDS

CR 2000-237782 [20]

AB WO 200012535 A UPAB: 20000630

NOVELTY - An isolated *Neisseria meningitidis* NMASP polypeptide, which has a molecular weight of about 40-55 kD, determined by sodium dodecyl sulfate (SDS)-PAGE (polyacrylamide gel electrophoresis), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a peptide fragment of NMASP;
- (2) an isolated antibody that specifically binds NMASP;
- (3) an antigenic composition, vaccine or pharmaceutical composition comprising NMASP or a peptide fragment or an antibody of (2);
- (4) an isolated DNA comprising a nucleotide sequence encoding NMASP or its fragments;
- (5) an isolated DNA sequence having a 153 base pair (bp) sequence given in the specification;
- (6) an isolated DNA which comprises a nucleotide sequence that hybridizes under high stringency conditions to a sequence of (5);
- (7) plasmid pNmAH116 obtainable from *Escherichia coli* Top10 pNmAH116) as deposited with the ATCC and assigned accession number 98839;
- (8) a method (A) for assaying for an agent that interacts with NMASP;
- (9) an antagonist which inhibits the activity or expression of NMASP; and
- (10) a method for identifying compounds which interact with and inhibitor or activate an activity of NMASP, comprising contacting the polypeptide with the compound to be screened under interaction conditions and assessing the interaction, an interaction being

Searcher : Shears 308-4994

09/7388090

associated with a second component capable of providing a signal in the presence or absence of a signal generated by the interaction between the polypeptide and the compound.

ACTIVITY - Antibacterial; Anti-inflammatory.

MECHANISM OF ACTION - Vaccine.

USE - NMASP can be used in a method to produce an immune response in an animal. The sequences and antibodies are useful for protection against *N. meningitidis*, the most common cause of bacterial meningitidis and septicemia in infants and young adults. The antibody is a cytotoxic antibody that mediates complement killing of *N. meningitidis*. NMASP and NMASP-derived polypeptides may be used as ligands to detect antibodies elicited in response to *N. meningitidis* infections.

ADVANTAGE - Antibody generated against the NMASP polypeptide in an animal host will exhibit bactericidal and/or opsonic activity against many *Neisseria meningitidis* strains and thus confer broad cross-strain protection. Bactericidal and/or opsonic antibody will prevent the bacterium from infecting the host and/or enhance the clearance of the pathogen by the host's immune system.

Dwg.0/2

L8 ANSWER 4 OF 24 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-237782 [20] WPIDS
CROSS REFERENCE: 2000-256581 [20]
DOC. NO. NON-CPI: N2000-178293
DOC. NO. CPI: C2000-072442
TITLE: Non-cytosolic NGSP polypeptide and
polynucleotide sequence from *Neisseria*
useful for diagnosis, prevention or treatment of
Neisseria infections.
DERWENT CLASS: B04 C06 C07 D16 S03
INVENTOR(S): HARRIS, A M; JACKSON, W J
PATENT ASSIGNEE(S): (ANTE-N) ANTEX BIOLOGICS INC
COUNTRY COUNT: 85
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG

WO 2000012133	A1	20000309	(200020)*	EN	68
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC					
MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES					
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK					
LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG					
SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW					
AU 9959066	A	20000321	(200031)		

APPLICATION DETAILS:

Searcher : Shears 308-4994

09/7388090

PATENT NO	KIND	APPLICATION	DATE
WO 2000012133	A1	WO 1999-US20070	19990901
AU 9959066	A	AU 1999-59066	19990901

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9959066	A Based on	WO 200012133

PRIORITY APPLN. INFO: US 1998-98685 19980901

AN 2000-237782 [20] WPIDS

CR 2000-256581 [20]

AB WO 200012133 A UPAB: 20000630

NOVELTY - Isolated NGSP **polypeptide** (I) of **Neisseria** spp. but not from **N. meningitidis** has a molecular weight of 40-55 kD determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (**SDS-PAGE**).

The NGSP **polypeptide** is the whole, or a subunit of a non-cytosolic **protein** embedded in or located in the bacterial envelope which includes the inner membrane, outer surface and periplasmic space.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a peptide fragment (II) of (I);
- (2) an antibody (III) that specifically binds (I) or a fragment of (I);
- (3) an antigenic, pharmaceutical or vaccine composition comprising (I) or (II) and a carrier or diluent;
- (4) a pharmaceutical composition comprising (III);
- (5) an isolated **DNA** (IV) comprising a **nucleotide** sequence encoding (I), (II) or a fragment of these which has the defined sequence of 153, 1242, 1395, 69 or 46 base pairs given in the specification;
- (6) an isolated **DNA** comprising a **nucleotide** sequence which hybridizes under high stringency conditions to (IV) or its complement;
- (7) plasmid pTLZ-NgHtrA number 2 obtainable from *Escherichia coli* JM109 (pTLZ-NgHtrA number 2) (ATCC PTA-470);
- (8) an antagonist which inhibits the activity or expression of (I);
- (9) a method for identifying compounds which interact with, inhibit or activate an activity of (I) comprising contacting a composition comprising (I) with the candidate compound (A) to permit interaction between (A) and (I); (A) is associated with a second component capable of providing a detectable signal in response to interaction of (I) with (A) so that the presence or absence of a

Searcher : Shears 308-4994

signal generated from the interaction is determined; and

(10) a method for assaying for an agent that interacts with (I) which can be used as a diagnostic, prophylactic or therapeutic agent against Neisseria infection comprising:

(i) contacting a cell expressing (I) with an agent labeled with a detectable marker for a sufficient length of time to allow interaction;

(ii) washing the cells; and

(iii) detecting any marker associated with the cells indicating that the agent interacts with (I).

ACTIVITY - Antibacterial.

No biological data given.

MECHANISM OF ACTION - Vaccine.

(I) has conserved Arg-Gly-Asp and Arg-Gly-Asn groups near the C-terminus which function as adherence domains for extracellular matrix proteins. Using (I) as a vaccine produces antibodies which inhibit (I) binding to the host's cellular matrix reducing attachment and/or subsequent invasion.

USE - (I) and (II) can be used to immunize an animal and produce an immune response (claimed). (I) and (II) can be used as ligands to detect antibodies elicited in response to Neisseria infections and also as antigens or immunogens for inducing Neisseria-specific antibodies which are useful in immunoassays to detect Neisseria in biological specimens. (IV) can be used as probes to identify Neisseria in biological specimens by hybridization or polymerase chain reaction amplification. (I) can also be used in screening assays to identify agents and compounds which useful as diagnostic, prophylactic or therapeutic agents against Neisseria infection (claimed).

Dwg.0/2

L8 ANSWER 5 OF 24 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
 ACCESSION NUMBER: 2000-147612 [13] WPIDS
 DOC. NO. NON-CPI: N2000-109219
 DOC. NO. CPI: C2000-046316
 TITLE: Generation of cell lines and clones specific to a particular protein for screening antigenic peptides which are used as vaccines in treating meningococcal, gonococcal infections.
 DERWENT CLASS: B04 D16 S03
 INVENTOR(S): ALA'ALDEEN, D; TODD, I
 PATENT ASSIGNEE(S): (UYNO-N) UNIV NOTTINGHAM
 COUNTRY COUNT: 86
 PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000003003	A2	20000120	(200013)*	EN	51
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC					
Searcher			:	Shears	308-4994

09/7388090

MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK
LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG
SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
AU 9947917 A 20000201 (200028)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 200003003	A2	WO 1999-GB2205	19990709
AU 9947917	A	AU 1999-47917	19990709

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9947917	A Based on	WO 200003003

PRIORITY APPLN. INFO: GB 1998-14902 19980710

AN 2000-147612 [13] WPIDS

AB WO 200003003 A UPAB: 20000313

NOVELTY - Generation of T-cell lines and clones (C) specific to neisserial proteins (P) is new and comprises isolating peripheral blood mononuclear cells (PBMCs) from the peripheral blood of normal donors and patients recovering from neisserial disease, culturing the PBMCs with (P), stimulating proliferation of T cell lines and clones and maintaining the same by regular proliferation stimulation.

DETAILED DESCRIPTION - Generation of T-cell lines and clones (C) specific to neisserial proteins (P) is new and comprises isolating peripheral blood mononuclear cells (PBMCs) from the peripheral blood of normal donors and patients recovering from neisserial disease, culturing the PBMCs with (P) with or without a proliferation stimulant, stimulating proliferation of T cell lines and clones which are specific to neisserial proteins and maintaining the same by regular stimulation.

INDEPENDENT CLAIMS are also included for the following:

(1) a method (II) of detecting CD4+T-cell (T) stimulating proteins, by fractionating (P) and testing their ability to proliferate (C);

(2) a method (III) and (IV) of detecting CD4+ T cell stimulating recombinant proteins and peptides by screening genomic meningococcal or gonococcal expression library (L) and their phage display libraries (PDLs) respectively;

(3) a method of detecting CD4+T-cell stimulating peptides comprising screening meningococcal or gonococcal genomic phage display libraries (PDLs) to identify peptides which stimulate T-cell

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lines and clones;

(4) use of a polypeptide in the manufacture of a vaccine (V) against neisserial disease comprising an 880 amino acid sequence (fully defined in the specification)

(5) CD4+ stimulant polypeptide comprising 880 amino acids (aa) or 548 aa;

(6) **nucleotides** sequence comprising 2761 base pairs (bp) or 1647 bp or their active derivatives;

(7) vaccine comprising (5); and

(8) a purified and isolated **DNA** composition comprising (6).

ACTIVITY - Antibacterial; anti-inflammatory

MECHANISM OF ACTION - Vaccine.

USE - (I) is used to generate (C) which are used in (II, III and IV) to identify CD4+ T-cell stimulants. The polypeptide and composition comprising peptides having 880 aa or 548 aa are used as vaccines for treating neisserial diseases by inducing T-cell proliferation (claimed). **DNA** construct comprising 2761 bp or 1647 bp is used in the manufacture of medicament for treating neisserial diseases (claimed) like meningitis, gonorrhoea, overwhelming septicemia, septic arthritis.

ADVANTAGE - The peptide epitope that stimulates specific CD4+ T-cells is identified and characterized. The vaccine includes non-capsular antigens that stimulate T-cell memory and thus a long lasting, cross protective immunity is generated.

Dwg.0/3

L8	ANSWER 6 OF 24	MEDLINE	DUPLICATE 1
ACCESSION NUMBER:	2000420826	MEDLINE	
DOCUMENT NUMBER:	20378984		
TITLE:	Two glycosylase/abasic lyases from Neisseria mucosa that initiate DNA repair at sites of UV-induced photoproducts.		
AUTHOR:	Nyaga S G; Lloyd R S		
CORPORATE SOURCE:	Center for Molecular Science, the University of Texas Medical Branch, Galveston, Texas 77555, USA.		
CONTRACT NUMBER:	ES04091 (NIEHS) ES06676 (NIEHS)		
SOURCE:	JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Aug 4) 275 (31) 23569-76. Journal code: HIV. ISSN: 0021-9258.		
PUB. COUNTRY:	United States Journal; Article; (JOURNAL ARTICLE)		
LANGUAGE:	English		
FILE SEGMENT:	Priority Journals; Cancer Journals		
ENTRY MONTH:	200011		
ENTRY WEEK:	20001102		
AB	Diverse organisms ranging from Escherichia coli to humans contain a variety of DNA repair proteins that function in		
	Searcher	:	Shears 308-4994

the removal of damage caused by shortwave UV light. This study reports the identification, purification, and biochemical characterization of two DNA glycosylases with associated abasic lyase activity from *Neisseria mucosa*. These enzymes, pyrimidine dimer glycosylase I and II (Nmu-pdg I and Nmu-pdg II), were purified 30,000- and 10,000-fold, respectively.

SDS-polyacrylamide gel electrophoresis

analysis indicated that Nmu-pdg I is approximately 30 kDa, whereas Nmu-pdg II is approximately 19 kDa. The N-terminal amino acid sequence of Nmu-pdg II exhibits 64 and 66% identity with *E. coli* and *Hemophilus parainfluenzae* endonuclease III, respectively. Both Nmu-pdg I and Nmu-pdg II were found to have broad substrate specificities, as evidenced by their ability to incise DNA containing many types of UV and some types of oxidative damage. Consistent with other glycosylase/abasic lyases, the existence of a covalent enzyme-DNA complex could be demonstrated for both Nmu-pdg I and II when reactions were carried out in the presence of sodium borohydride. These data indicate the involvement of an amino group in the catalytic reaction mechanism of both enzymes.

L8 ANSWER 7 OF 24 MEDLINE DUPLICATE 2
 ACCESSION NUMBER: 2000278119 MEDLINE
 DOCUMENT NUMBER: 20278119
 TITLE: Construction and characterization of *Haemophilus ducreyi* lipooligosaccharide (LOS) mutants defective in expression of heptosyltransferase III and beta1,4-glucosyltransferase: identification of LOS glycoforms containing lactosamine repeats.
 AUTHOR: Filiatrault M J; Gibson B W; Schilling B; Sun S; Munson R S Jr; Campagnari A A
 CORPORATE SOURCE: Department of Microbiology, University at Buffalo, Buffalo, New York 14214, USA.
 CONTRACT NUMBER: RO1 AI30006 (NIAID)
 RO1 AI38444 (NIAID)
 RO1 AI31254 (NIAID)
 +
 SOURCE: INFECTION AND IMMUNITY, (2000 Jun) 68 (6) 3352-61.
 Journal code: GO7. ISSN: 0019-9567.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; Cancer Journals
 OTHER SOURCE: GENBANK-AF215936
 ENTRY MONTH: 200009
 ENTRY WEEK: 20000901

AB To begin to understand the role of the lipooligosaccharide (LOS) molecule in chancroid infections, we constructed mutants defective in expression of glycosyltransferase genes. Pyocin lysis and immunoscreening was used to identify a LOS mutant of *Haemophilus*

Searcher : Shears 308-4994

ducreyi 35000. This mutant, HD35000R, produced a LOS molecule that lacked the monoclonal antibody 3F11 epitope and migrated with an increased mobility on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Structural studies indicated that the principal LOS glycoform contains lipid A, Kdo, and two of the three core heptose residues. HD35000R was transformed with a plasmid library of *H. ducreyi* 35000 DNA, and a clone producing the wild-type LOS was identified. Sequence analysis of the plasmid insert revealed one open reading frame (ORF) that encodes a protein with homology to the WaaQ (heptosyltransferase III) of *Escherichia coli*. A second ORF had homology to the LgtF (glucosyltransferase) of *Neisseria meningitidis*. Individual isogenic mutants lacking expression of the putative *H. ducreyi* heptosyltransferase III, the putative glucosyltransferase, and both glycosyltransferases were constructed and characterized. Each mutant was complemented with the representative wild-type genes in trans to restore expression of parental LOS and confirm the function of each enzyme. Matrix-assisted laser desorption ionization mass spectrometry and SDS-PAGE analysis identified several unique LOS glycoforms containing di-, tri-, and poly-N-acetyllactosamine repeats added to the terminal region of the main LOS branch synthesized by the heptosyltransferase III mutant. These novel *H. ducreyi* mutants provide important tools for studying the regulation of LOS assembly and biosynthesis.

L8 ANSWER 8 OF 24 BIOSIS COPYRIGHT 2001 BIOSIS
 ACCESSION NUMBER: 2000:202759 BIOSIS
 DOCUMENT NUMBER: PREV200000202759
 TITLE: Cloning and characterization of the
 lipooligosaccharide galactosyltransferase II gene of
Haemophilus ducreyi.
 AUTHOR(S): Sun, Shuhua; Schilling, Birgit; Tarantino, Laurie;
 Tullius, Michael V.; Gibson, Bradford W.; Munson,
 Robert S., Jr. (1)
 CORPORATE SOURCE: (1) Children's Research Institute, 700 Children's
 Dr., Room W402, Columbus, OH, 43205 USA
 SOURCE: Journal of Bacteriology, (April, 2000) Vol. 182, No.
 8, pp. 2292-2298.
 ISSN: 0021-9193.
 DOCUMENT TYPE: Article
 LANGUAGE: English
 SUMMARY LANGUAGE: English
 AB *Haemophilus ducreyi* is the etiologic agent of chancroid, a genital
 ulcer disease. The lipooligosaccharide (LOS) is considered to be a
 major virulence determinant and has been implicated in the adherence
 of *H. ducreyi* to keratinocytes. Strain A77, an isolate from the
 Paris collection, is serum sensitive, poorly adherent to
 fibroblasts, and deficient in microcolony formation. Structural
 Searcher : Shears 308-4994

analysis indicates that the LOS of strain A77 lacks the galactose residue found in the N-acetyllactosamine portion of the strain 35000HP LOS as well as the sialic acid substitution. From an *H. ducreyi* 35000HP genomic DNA library, a clone complementing the defect in A77 was identified by immunologic screening with monoclonal antibody (MAb) 3F11, a MAb which recognizes the N-acetyllactosamine portion of strain 35000HP LOS. The clone contained a 4-kb insert that was sequenced. One open reading frame which encodes a protein with a molecular weight of 33,400 was identified. This protein has homology to glycosyltransferases of *Haemophilus influenzae*, *Haemophilus somnus*, *Neisseria* species, and *Pasteurella haemolytica*. The putative *H. ducreyi* glycosyltransferase gene was insertionally inactivated, and an isogenic mutant of strain 35000HP was constructed. The most complex LOS glycoform produced by the mutant has a mobility on sodium dodecyl sulfate-polyacrylamide gel identical to that of the LOS of strain A77 and lacks the 3F11-binding epitope. Structural studies confirm that the most complex glycoform of the LOS isolated from the mutant lacks the galactose residue found in the N-acetyllactosamine portion of the strain 35000HP LOS. Although previously published data suggested that the serum-sensitive phenotype of A77 was due to the LOS mutation, we observed that the complemented A77 strain retained its serum-sensitive phenotype and that the galactosyltransferase mutant retained its serum-resistant phenotype. Thus, the serum sensitivity of strain A77 cannot be attributed to the galactosyltransferase mutation in strain A77.

L8 ANSWER 9 OF 24 MEDLINE DUPLICATE 3
 ACCESSION NUMBER: 2000005611 MEDLINE
 DOCUMENT NUMBER: 20005611
 TITLE: *Neisseria gonorrhoeae* bacterioferritin: structural heterogeneity, involvement in iron storage and protection against oxidative stress.
 AUTHOR: Chen C Y; Morse S A
 CORPORATE SOURCE: Division of AIDS, Sexually Transmitted Diseases and Tuberculosis Laboratory Research, National Centers for Infectious Disease, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.. cycl@cdc.gov
 SOURCE: MICROBIOLOGY, (1999 Oct) 145 (Pt 10) 2967-75.
 Journal code: BXW. ISSN: 1350-0872.
 PUB. COUNTRY: ENGLAND: United Kingdom
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-U76633; GENBANK-U76634
 ENTRY MONTH: 200004
 ENTRY WEEK: 20000403
 AB The iron-storage protein bacterioferritin (Bfr) from *Neisseria gonorrhoeae* strain F62 was identified in cell-free
 Searcher : Shears 308-4994

extracts and subsequently purified by column chromatography. Gonococcal Bfr had an estimated molecular mass of 400 kDa by gel filtration; however, analysis by **SDS-PAGE** revealed that it was composed of 18 kDa (BfrA) and 22 kDa (BfrB) subunits. **DNA** encoding BfrB was amplified by PCR using degenerate primers derived from the N-terminal amino acid sequence of BfrB and from a C-terminal amino acid sequence of *Escherichia coli* Bfr. The **DNA** sequence of bfrA was subsequently obtained by genome walking using single-specific-primer PCR. The two Bfr genes were located in tandem with an intervening gap of 27 bp. A potential Fur-binding sequence (12 of 19 bp identical to the consensus neisserial fur sequence) was located within the 5' flanking region of bfrA in front of a putative -35 hexamer. The homology between the **DNA** sequences of bfrA and bfrB was 55.7%; the deduced amino acid sequences of BfrA (154 residues) and BfrB (157 residues) showed 39.7% identity, and showed 41.3% and 56.1% identity, respectively, to *E. coli* Bfr. Expression of recombinant BfrA and BfrB in *E. coli* strain DH5alpha was detected on Western blots probed with polyclonal anti-*E. coli* Bfr antiserum. Most Bfrs are homopolymers with identical subunits; however, the evidence presented here suggests that gonococcal Bfr was composed of two similar but not identical subunits, both of which appear to be required for the formation of a functional Bfr. A Bfr-deficient mutant was constructed by inserting the omega fragment into the BfrB gene. The growth of the BfrB-deficient mutant in complex medium was reduced under iron-limited conditions. The BfrB-deficient mutant was also more sensitive to killing by H₂O₂ and paraquat than the isogenic parent strain. These results demonstrate that gonococcal Bfr plays an important role in iron storage and protection from iron-mediated oxidative stress.

L8 ANSWER 10 OF 24 TOXLINE

ACCESSION NUMBER: 1999:48657 TOXLINE

DOCUMENT NUMBER: CRISP-99-AI43568-01

TITLE: **DNA** SEQUENCE OF STAPHYLOCOCCUS AUREUS 8325
GENOME.

AUTHOR: IANDOLO J J

CORPORATE SOURCE: OKLAHOMA UNIV HLTH SCIS CTR, PO BOX 26901, OKLAHOMA
CITY, OK 73190
U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC
HEALTH SERVICE; NATIONAL INST. OF HEALTH, NATIONAL
INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES.

CONTRACT NUMBER: 1R01AI43568-01

SOURCE: (1998). Crisp Data Base National Institutes Of
Health. Award Type: G = Grant

PUB. COUNTRY: United States

DOCUMENT TYPE: (RESEARCH)

FILE SEGMENT: CRISP

LANGUAGE: English

Searcher : Shears 308-4994

ENTRY MONTH: 199904

AB RPROJ/CRISP In 1995, Fleischman et al. (40) determined the **nucleotide** sequence of *Haemophilus influenzae* Rd without prior restriction site mapping and cosmid cloning. This was a landmark event, signaling that automated **DNA** sequencing and analysis have matured into robust, highly efficient technologies promising to revolutionize the biological sciences. An important breakthrough that will greatly facilitate closure and proof-reading of the sequence which was reported by Dr. Mike Hunkapiller from ABI and Dr. Roe at the recent September Hilton Head **DNA** Sequencing conference was the results of experiments in which we successfully obtained sequence data in excess of 450 bases directly from several bacterial genomes. The genomic sequence of several other procaryotes have or will be completed shortly, including our own genomic sequencing of the important human pathogens *Neisseria gonorrhoeae* and *Streptococcus pyogenes*. Collectively, these studies will considerably enhance our understanding of procaryotic molecular biological processes, and provide new avenues for gene discovery and comparative genetics. From a practical standpoint, procaryotic genome sequencing will enhance our ability to understand processes occurring during the pathogenesis of infectious disease. These studies should provide new approaches for drug discovery, a necessity of increasing importance as microbial antibiotic resistance threatens our ability to treat bacterial infections. In this application, we propose to determine the **nucleotide** sequence of the genome of *Staphylococcus aureus*. This organism is a potent pathogen widely found in the human and animal environment. It is capable of producing upwards of 34 different extracellular **proteins** most of which have been shown to play a role in the pathogenicity of the organism or to enhance virulence. Although the incidence of infection in the general population is not well documented, it is generally accepted that *S. aureus* accounts for up to one-third of all nosocomial bacteremia. Other than the details of abscess formation, very little is actually of the pathogenesis of staphylococcal disease. Understanding the circuits (*agr*, *sar* and probably others) which control the expression of virulence related genes is paramount to understanding the genetic response of the organism to host generated signals. We propose that acquisition of the sequence of the genome of the *Staphylococcus aureus* strain 8325 will greatly facilitate understanding the mechanism of disease produced by this organism and closely related species. Therefore, as a single specific aim, we will sequence and annotate the 2.8 Mb genome of this important pathogen.

L8 ANSWER 11 OF 24 MEDLINE

DUPLICATE 4

ACCESSION NUMBER: 1998136610 MEDLINE

DOCUMENT NUMBER: 98136610

TITLE: Molecular, genetic, and functional analysis of
 Searcher : Shears 308-4994

AUTHOR: homozygous C8 beta-chain deficiency in two siblings.
 KORPNATE SOURCE: Kotnik V; Luznik-Bufon T; Schneider P M; Kirschfink M
 Institute of Microbiology and Immunology, University
 of Ljubljana, Slovenia.. vkotnik@ibmi.mf.uni-lj.si
 SOURCE: IMMUNOPHARMACOLOGY, (1997 Dec) 38 (1-2) 215-21.
 Journal code: GY3. ISSN: 0162-3109.
 PUB. COUNTRY: Netherlands
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199806
 ENTRY WEEK: 19980604

AB C8 deficiency is associated with an increased susceptibility to
 neisserial infections. We present a case of an 11 year old boy who
 suffered from infection with *Neisseria meningitidis*.
 Medical history of the patient and his family (n = 5) did not
 indicate any previous immunodeficiency symptoms. Results from the
 analysis of phagocyte and lymphocyte functions were within the
 normal range. No hemolytic activities of the classical (CH50) and
 the alternative (APH50) pathways of complement were measurable, and
 SC5b-9 **protein** complexes could not be detected in the
 patient's plasma. Further analysis by highly sensitive ELISA and
 functional assays revealed a complete deficiency of C8. Upon the
 reconstitution with purified C8 total hemolytic activity could be
 restored. **SDS-PAGE** and Western blot analysis
 established a deficiency of the C8 beta chain. Genetic analysis at
 the genomic **DNA** level demonstrated the common C-T mutation
 in exon 9 of the C8B gene. Family analysis presented the older
 sister with non-detectable function of C8 in serum, both parents
 with about half-normal C8 titres, and the younger sister with normal
 C8 function. The parents and both sisters were asymptomatic,
 although the older of the sisters presented with the same complete
 C8 beta-chain deficiency as the patient described. In conclusion:
 the common C-T mutation in the C8B genes is the genetic basis of C8
 beta-chain deficiency in two members of this Bosnian family.

L8 ANSWER 12 OF 24 MEDLINE DUPLICATE 5
 ACCESSION NUMBER: 1998353647 MEDLINE
 DOCUMENT NUMBER: 98353647
 TITLE: The aroQ and pheA domains of the bifunctional
 P-protein from *Xanthomonas campestris* in a context of
 genomic comparison.
 AUTHOR: Gu W; Williams D S; Aldrich H C; Xie G; Gabriel D W;
 Jensen R A
 CORPORATE SOURCE: Department of Microbiology and Cell Science,
 University of Florida, Gainesville, USA.
 SOURCE: MICROBIAL AND COMPARATIVE GENOMICS, (1997) 2 (2)
 141-58.
 Journal code: C5D. ISSN: 1090-6592.
 Searcher : Shears 308-4994

09/7388090

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U64518
ENTRY MONTH: 199810
ENTRY WEEK: 19981004

AB The gene (denoted *aroQp.pheA*) encoding the bifunctional P-**protein** (chorismate mutase-P/prephenate dehydratase) from *Xanthomonas campestris* was cloned. *aroQp.pheA* is essential for L-phenylalanine biosynthesis. DNA sequencing of the smallest subclone capable of functional complementation of an *Escherichia coli* phenylalanine auxotroph revealed a putative open reading frame (ORF) of 1200 bp that would encode a 43,438-Da **protein**. AroQp.PheA exhibited 51% amino acid identity with a *Pseudomonas stutzeri* homologue and greater than 30% identities with AroQp.PheA **proteins** from *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and a number of enteric bacteria. AroQp.PheA from *X. campestris*, when expressed in *E. coli*, possesses a 40-residue amino-terminal extension that is lysine-rich and that is absent in all of the AroQp.PheA homologues known at present. About 95% of AroQp.PheA was particulate and readily sedimented by low-speed centrifugation. Soluble preparations of cloned AroQp.PheA exhibited a native molecular mass of 81,000 Da, indicating that the active enzyme species is a homodimer. These preparations were unstable after purification of about 40-fold, even in the presence of glycerol, which was an effective protectant before fractionation. When AroQp.PheA was overproduced by a T7 translation vector, unusual inclusion bodies having a macromolecular structure consisting of **protein** fibrils were observed by electron microscopy. Insoluble **protein** collected at low-speed centrifugation possessed high catalytic activity. The single band obtained via SDS-PAGE was used to confirm the translational start via N-terminal amino acid sequencing. A perspective on the evolutionary relationships of monofunctional AroQ and PheA **proteins** and the AroQp.PheA family of **proteins** is presented. A *serC* gene located immediately upstream of *X. campestris aroQp.pheA* appears to reflect a conserved gene organization, and both may belong to a single transcriptional unit.

L8 ANSWER 13 OF 24 MEDLINE

DUPLICATE 6

ACCESSION NUMBER: 97039866 MEDLINE

DOCUMENT NUMBER: 97039866

TITLE: Proline iminopeptidase gene from *Xanthomonas campestris* pv. *citri*.

AUTHOR: Alonso J; Garcia J L

CORPORATE SOURCE: Departamento de Microbiologia Molecular, Consejo Superior de Investigaciones Cientificas, Velazquez, Madrid, Spain.

Searcher : Shears 308-4994

SOURCE: MICROBIOLOGY, (1996 Oct) 142 (Pt 10) 2951-7.
Journal code: BXW. ISSN: 1350-0872.

PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-Z54150

ENTRY MONTH: 199703

AB The pip gene coding for the proline iminopeptidase (Pip) of *Xanthomonas campestris* pv. *citri* was cloned in an *Escherichia coli* leuB strain using a selective medium containing the dipeptide D-Ala-L-Leu as the sole source of L-leucine. **Nucleotide** sequencing of this gene revealed a 939 bp open reading frame encoding a 312 amino acid **protein** (35 126 Da). The deduced amino acid sequence showed 47% identity with the Pip from *Neisseria gonorrhoeae*. A lacZ-pip fusion gene was overexpressed in *E. coli* under the control of the Plac promoter. The Pip of *X. campestris* hydrolysed L-prolyl-p-nitroanilide with the highest efficiency, but was also able to hydrolyse L-alanyl-p-nitroanilide and D-alanyl-p-nitroanilide. The molecular mass of Pip was found to be 35 kDa by **SDS-PAGE** and 120 kDa by gel filtration, suggesting that the active enzyme is a multimer.

L8 ANSWER 14 OF 24 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 96258475 EMBASE

DOCUMENT NUMBER: 1996258475

TITLE: [Molecular epidemiology of meningitis due to *Neisseria meningitidis* in Mali: Isolation of a new variant (P1.y) of the class 1 **protein**].
EPIDEMIOLOGIE MOLECULAIRE DE LA MENINGITE A MENINGOCOQUE AU MALI: ISOLEMENT D'UN NOUVEAU VARIANT (P1.Y) DE LA PROTEINE DE CLASSE 1.

AUTHOR: Koumare B.; Achtman M.; Bougoudogo F.; Cisse M.; Wang J.F.

CORPORATE SOURCE: Inst. Nat. de Rech. en Sante Publ., Service de Bacteriologie-Virologie, BP 1771, Bamako, Mali

SOURCE: Bulletin of the World Health Organization, (1996) 74/4 (375-379).
ISSN: 0042-9686 CODEN: BWHOA6

COUNTRY: Switzerland

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology
008 Neurology and Neurosurgery
017 Public Health, Social Medicine and Epidemiology

LANGUAGE: French

SUMMARY LANGUAGE: English; French

Searcher : Shears 308-4994

AB The study deals with 570 strains of Neisseriaceae isolated between 1989 and 1994 in Mali: 396 of the strains were isolated from samples of cerebrospinal fluid and 174 from the throat. Serogroup C accounted for 55% of all strains. Antigenic structure was determined by ELISA, SDS-PAGE and transfer to nitrocellulose membrane for immunoblotting with monoclonal antibodies produced at the Max Planck Institute for Molecular Genetics. For serogroup A, the class 1 protein types found were P1.7 for strains isolated prior to 1994 and P1.9 for strains isolated in 1994. P1.7 is specific to clone IV-1 and P1.9 to clone III-1, which was responsible for the 1994 epidemic. All strains of serogroup C isolated from fluid CSF and most strains isolated from the throat exhibit a new type of class 1 protein which the authors have designated P1.y. P1.y is characteristic of Malian strains of serogroup C; it is rare or absent in strains from other countries (Burkina Faso, Ghana, Italy, USA). The nucleotide sequence of the gene expressing P1.y and the corresponding amino acid sequence were determined at the National Institute for Biological Standards and Control, England.

L8 ANSWER 15 OF 24 MEDLINE

ACCESSION NUMBER: 96423180 MEDLINE
 DOCUMENT NUMBER: 96423180
 TITLE: Identification and characterization of genes required for post-translational modification of Campylobacter coli VC167 flagellin.
 AUTHOR: Guerry P; Doig P; Alm R A; Burr D H; Kinsella N; Trust T J
 CORPORATE SOURCE: Enteric Diseases Program, Naval Medical Research Institute, Bethesda, Maryland 20889, USA..
 guerry_p@mail2.nmri.nmmc.navy.mil
 SOURCE: MOLECULAR MICROBIOLOGY, (1996 Jan) 19 (2) 369-78.
 Journal code: MOM. ISSN: 0950-382X.
 PUB. COUNTRY: ENGLAND: United Kingdom
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-U25992
 ENTRY MONTH: 199702

AB Two genes have been identified in Campylobacter coli VC167 which are required for the biosynthesis of post-translational modifications on flagellin proteins. The ptmA gene encodes a protein of predicted M(r) 28,486 which shows significant homology to a family of alcohol dehydrogenases from a variety of bacteria. The ptmB gene encodes a protein of predicted M(r) 26,598 with significant homology to CMP-N-acetylneuraminic acid synthetase enzymes involved in sialic acid capsular biosynthesis in Neisseria meningitidis and Escherichia coli K1. Site-specific mutation of either ptmA or ptmB caused loss of

Searcher : Shears 308-4994

reactivity with antisera specific to the post-translational modifications and a change in the isoelectric focusing fingerprints relative to the parent strains. Mutation of *ptmB*, but not of *ptmA*, caused a change in apparent *M(r)* of the flagellin subunit in **SDS-PAGE** gels. The *ptmA* and *ptmB* genes are present in other strains of *Campylobacter*. In a rabbit model the *ptmA* mutant showed a reduced ability to elicit protection against subsequent challenge with heterologous strains of the same Lior serotype compared to the parental wild-type strain. This suggests that the surface-exposed post-translational modifications may play a significant role in the protective immune response.

L8 ANSWER 16 OF 24 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1996:267378 BIOSIS

DOCUMENT NUMBER: PREV199698823507

TITLE: Tandem repeats of the tetramer 5'-CAAT-3' present in *lic2A* are required for phase variation but not lipopolysaccharide biosynthesis in *Haemophilus influenzae*.

AUTHOR(S): High, Nicola J. (1); Jennings, Michael P.; Moxon, E. Richard

CORPORATE SOURCE: (1) 1.800, Stopford Building, Sch. Biol. Sci., Univ. Manchester, Manchester M13 9PT UK

SOURCE: Molecular Microbiology, (1996) Vol. 20, No. 1, pp. 165-174.
ISSN: 0950-382X.

DOCUMENT TYPE: Article

LANGUAGE: English

AB A novel lipopolysaccharide (LPS) biosynthesis gene, *lic2B*, which is required for the biosynthesis of a phase-variable LPS structure expressed by *Haemophilus influenzae* RM7004 is described. The product of this gene is homologous to *Lic2A* and the recently described LPS biosynthetic enzymes, *LgtB* from *Neisseria gonorrhoea* and *LgtE* from *Neisseria meningitidis*, and *LpsA* from *Pasteurella haemolytica*. Of this family of enzymes only *Lic2A* contains the repetitive tetrapeptide motif (SINQ)-*n* encoded by multiple tandem repeats of 5'-CAAT-3'. This observation suggested that (SINQ)-*n* might not be a prerequisite for the catalytic activity of this **protein**. To address this hypothesis, we deleted the 5'-CAAT-3' repeats from *lic2A* so that the **protein** encoded by the modified gene was analogous to *Lic2B*. This mutation had no apparent effect on the overall apparent molecular weight of LPS as judged by Tricine-**SDS-PAGE** and did not affect ability to react with monoclonal antibody 4C4. It was therefore concluded that (SINQ)-*n* is not a prerequisite for the enzymatic function of *Lic2A* and that the 5'-CAAT-3' repeats in *lic2A* function solely as a mechanism for generating phase variation. This observation suggested that wide variation in the number of 5'-CAAT-3' repeats might be tolerated in *lic2A*, and this was

Searcher : Shears 308-4994

confirmed by surveying the number of 5'-CAAT-3' repeats in a range of different *H. influenzae* strains. The predicted secondary structure of (SINQ)-*n* indicates that it forms a highly flexible random coiled structure, which is unlikely to impede formation of the domains that may be required for catalytic activity. This characteristic is also a feature of repetitive tetrapeptides encoded by other tetrameric repeats located within coding sequences present on the chromosome of *H. influenzae* Rd.

L8 ANSWER 17 OF 24 MEDLINE DUPLICATE 7
 ACCESSION NUMBER: 95198536 MEDLINE
 DOCUMENT NUMBER: 95198536
 TITLE: Lipooligosaccharide biosynthesis in *Neisseria gonorrhoeae*: cloning, identification and characterization of the alpha 1,5 heptosyltransferase I gene (*rfaC*) [published erratum appears in *Mol Microbiol* 1995 Apr;16(1):169].
 AUTHOR: Zhou D; Lee N G; Apicella M A
 CORPORATE SOURCE: Department of Microbiology, University of Iowa, Iowa City 52242..
 CONTRACT NUMBER: AI18384 (NIAID)
 AI24616 (NIAID)
 SOURCE: MOLECULAR MICROBIOLOGY, (1994 Nov) 14 (4) 609-18.
 Journal code: MOM. ISSN: 0950-382X.
 PUB. COUNTRY: ENGLAND: United Kingdom
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-U10385
 ENTRY MONTH: 199506

AB The identical partial deep-core structure of Hep alpha 1-3Hep alpha 1-5KDO in *Salmonella typhimurium* LT2 LPS and *Neisseria gonorrhoeae* LOS enabled us to isolate a DNA fragment from *N. gonorrhoeae* that was able to complement the alpha 1,5 LOS heptosyltransferase defect in the *S. typhimurium* *rfaC*630 (SA1377) mutant. SDS-PAGE analysis confirmed the production of wild-type LPS in the transformant. Subcloning revealed that complementation was due to a 1.2 kb fragment. Sequence analysis revealed a complete open reading frame capable of encoding a 36-37 kDa peptide. In vitro transcription-translation analysis of the 1.2 kb clone confirmed that a 37 kDa protein was encoded by this DNA fragment. The DNA sequence-deduced protein had 36% identity and 58% similarity to *S. typhimurium* heptosyltransferase I (*RfaC*). Primer extension analysis indicated that transcription of the cloned gene in *N. gonorrhoeae* strain 1291 begins 144 bp upstream of the start codon at a G nucleotide. An isogenic mutant of *N. gonorrhoeae* strain 1291 with an m-Tn3 insertion inside the coding sequence expressed a single truncated LOS with a similar molecular
 Searcher : Shears 308-4994

mass to *S. typhimurium* rfaC LPS. We conclude that the 1.2 kb fragment encodes the alpha 1,5 LOS heptosyltransferase I (RfaC) in *N. gonorrhoeae*. Our studies also provide further evidence that the third KDO residue in *S. typhimurium* LPS is added after the core synthesis is completed.

L8 ANSWER 18 OF 24 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1994:176611 BIOSIS

DOCUMENT NUMBER: PREV199497189611

TITLE: Genetic diversity of the iron-binding protein (Fbp) gene of the pathogenic and commensal

Neisseria.

AUTHOR(S): Genco, Caroline Attardo (1); Berish, Sally A.; Chen, Cheng-Yen; Morse, Stephen; Trees, David L.

CORPORATE SOURCE: (1) Dep. Microbiol. Immunol., Morehouse Sch. Med., Atlanta, GA 30310 USA

SOURCE: FEMS Microbiology Letters, (1994) Vol. 116, No. 2, pp. 123-129.

ISSN: 0378-1097.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The pathogenic *Neisseria* and most commensal

Neisseria species produce an iron-binding protein

(Fbp) when grown under iron-limited conditions. In the current study, we confirmed the presence of Fbp, as well as DNA sequences homologous to the gonococcal fbp, in strains of *V.*

gonorrhoeae, *N. meningitidis*, *N. cinerea*, *N. lactamica*, *N. subflava*, *N. kochii* and *N. polysaccharea*. The fbp genes from these strains were amplified by the polymerase chain reaction, digested with *StuI* or *RsaI*, and the restriction patterns examined. The patterns for the gonococcal and meningococcal fbp were virtually identical; however, variations were observed in the fbp sequences of the commensal *Neisseria* species. *N. flauescens*, *N. mucosa*, *N. sicca*, *N.*

ovis and *Branhamella catarrhalis*, did not produce Fbp as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and reactivity with an Fbp specific monoclonal antibody, nor did they hybridize to an fbp-specific DNA probe.

L8 ANSWER 19 OF 24 MEDLINE

DUPLICATE 8

ACCESSION NUMBER: 95058178 MEDLINE

DOCUMENT NUMBER: 95058178

TITLE: Expression of meningococcal epitopes in LamB of *Escherichia coli* and the stimulation of serosubtype-specific antibody responses.

AUTHOR: McCarvil J; McKenna A J; Grief C; Hoy C S; Sesardic D; Maiden M C; Feavers I M

CORPORATE SOURCE: Division of Bacteriology, National Institute for Biological Standards and Control, South Mimms, Hertfordshire, UK..

Searcher : Shears 308-4994

09/7388090

SOURCE: MOLECULAR MICROBIOLOGY, (1993 Oct) 10 (1) 203-13.
Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502

AB The class 1 outer membrane **protein** (OMP), a major variable surface antigen of *Neisseria meningitidis*, is a component of novel meningococcal vaccines currently in field trials. Serological variants of the **protein** are also used to serotype meningococci. Most of the amino acid changes that give rise to antigenic variants of the **protein** occur in two variable regions (VR1 and VR2) that are thought to form loops on the cell surface. The polymerase chain reaction (PCR) was used to amplify the **nucleotide** sequences encoding VR1 and VR2 from the chromosomal **DNA** of *N. meningitidis* strain M1080. These were cloned in frame into the lamB gene of the *Escherichia coli* expression vector pAJC264. Whole-cell enzyme-linked immunosorbent assays (ELISAs), using monoclonal antibodies, and **SDS-PAGE** confirmed that, upon induction, strains of *E. coli* carrying these constructs expressed hybrid LamB **proteins** containing the *N. meningitidis* surface loops. These strains were used to immunize rabbits and the resultant polyclonal antisera reacted specifically with the class 1 OMP of reference strain M1080 (P1.7). Immunogold labelling of meningococcal cells and whole-cell dot-blot analyses with these antisera showed that the variable epitopes were exposed on the cell surface and confirmed that this approach could be used to obtain serotype-specific antisera. The binding profiles of the antisera were determined from their reactions with overlapping synthetic **peptides** and their reactivity compared with that of relevant serotype-specific monoclonal antibodies. This approach was used successfully to raise antisera against two other class 1 OMP VR2s. A fourth antiserum raised against a VR2, including the P1.1 epitope, was not subtype specific.

L8 ANSWER 20 OF 24 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93273095 EMBASE
DOCUMENT NUMBER: 1993273095
TITLE: *Neisseria gonorrhoeae* strain MS11 harbouring a mutation in gene *aroA* is attenuated and immunogenic.
AUTHOR: Chamberlain L.M.; Strugnelli R.; Dougan G.; Hormaeche C.E.; Demarco de Hormaeche R.
CORPORATE SOURCE: Department of Pathology, Tennis Court Road, Cambridge CB2 1QP, United Kingdom
SOURCE: Microbial Pathogenesis, (1993) 15/1 (51-63).
ISSN: 0882-4010 CODEN: MIPAEV
COUNTRY: United Kingdom
Searcher : Shears 308-4994

DOCUMENT TYPE: Journal; Article
 FILE SEGMENT: 004 Microbiology
 013 Dermatology and Venereology
 026 Immunology, Serology and Transplantation
 037 Drug Literature Index

LANGUAGE: English

SUMMARY LANGUAGE: English

AB An aroA mutant of gonococcal strain MS11 was constructed (JKD298) and compared with the wild-type (JKD288). The requirement of JKD298 for aromatic compounds, typical of an aroA mutant, was demonstrated using defined media. Other than the expected auxotrophy, no further differences could be demonstrated between the parent and the aroA mutant. **SDS-PAGE** analysis of **protein** and lipopolysaccharide (LPS) profiles showed no differences between the strains. Bactericidal assays using human and guinea-pig normal sera showed that both strains were serum sensitive and were similarly converted to serum resistance by in vitro sialylation using CMP-NANA. Infectivity experiments in guinea-pig subcutaneous chambers showed considerably reduced virulence of the aroA JKD298, which could only infect chambers at very high doses. Established infections by either strain elicited a strong PMN response in which similar proportions of each strain were seen intracellularly. Infections by JKD298 provoked a strong antibody response as detected by ELISA using whole sonicated gonococci. This is the first demonstration of attenuation of *Neisseria gonorrhoeae* by introduction of a defined mutation in a metabolic gene.

L8 ANSWER 21 OF 24 TOXLIT

ACCESSION NUMBER: 1993:59032 TOXLIT

DOCUMENT NUMBER: CA-118-226653G

TITLE: Identification of meningococcal serosubtypes by polymerase chain reaction.

AUTHOR: Maiden MC J; Bygraves JA; McCarvil J; Feavers IM

CORPORATE SOURCE: Nal. Inst. Biol. Stand. Control, Hertsfordshire

SOURCE: J. Clin. Microbiol. (1992). Vol. 30, No. 11, pp. 2835-41.

CODEN: JCMIDW. ISSN. 0095-1137.

PUB. COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

FILE SEGMENT: CA

LANGUAGE: English

OTHER SOURCE: CA 118:226653

ENTRY MONTH: 199307

AB The polymerase chain reaction was used as the basis of a novel typing method for *Neisseria meningitidis*. Southern hybridization expts. demonstrated that it was possible to identify genes encoding different serol. variants of the meningococcal class 1 outer membrane **protein** by probing with polymerase chain reaction products corresponding to known epitopes. A set of 14

Searcher : Shears 308-4994

defined variable regions was prepd. in bacteriophage M13mp19 by the cloning of polymerase chain reaction products. The phage were dot blotted onto membrane filters, which were used as targets for hybridization of radiolabeled amplified class 1 outer membrane **protein** genes. Thus, the presence of many different subtype-specific epitopes could be investigated in one expt. This technique was evaluated with a set of serol. ref. strains, mainly of serogroup B organisms, and provided an alternative, rapid, and comprehensive typing system that was capable of distinguishing known serosubtypes and also of defining currently untypeable strains independently of **SDS-polyacrylamide gel electrophoresis** or serol. anal. An addnl. advantage of this technique was that in the case of an unknown serosubtype (i.e., one that did not hybridize with any of the known samples), the **DNA** amplified from the original sample could be used to det. the **nucleotide** sequence of the novel serosubtype and to clone the corresponding variable region into bacteriophage M13. It may be possible to develop this procedure for the diagnostic detection and typing of meningococci directly from clin. samples even when culture is not possible because of antibiotic treatment of an acute case.

L8 ANSWER 22 OF 24 MEDLINE

ACCESSION NUMBER: 91346008 MEDLINE

DOCUMENT NUMBER: 91346008

TITLE: Characterization of a soluble ferric reductase from *Neisseria gonorrhoeae*.

AUTHOR: Le Faou A E; Morse S A

CORPORATE SOURCE: Division of Sexually Transmitted Diseases Laboratory Research, Centers for Disease Control, Atlanta, Georgia 30333.

SOURCE: BIOLOGY OF METALS, (1991) 4 (2) 126-31.
Journal code: AU2. ISSN: 0933-5854.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199112

AB An NADH-dependent ferric reductase was identified in extracts of *Neisseria gonorrhoeae*. Enzyme activity was measured in an assay using ferrozine as the ferrous iron acceptor. Ferric reductase activity was enhanced by Mg²⁺ and flavine **nucleotides**. The enzyme reduced both citrate- and diphosphate-bound ferric iron as well as ferric hydroxide (Imferon). However, no activity was observed with either 30%-iron-saturated transferrin or with the gonococcal iron-binding **protein**, Fbp. The ferric reductase was found primarily within the cytoplasmic cell fraction. The soluble ferric reductase was purified 110-fold by ammonium sulfate precipitation, gel and anion-exchange chromatography. Results

Searcher : Shears 308-4994

obtained following gel chromatography and SDS/
polyacrylamide gel electrophoresis suggested that
the enzyme had a molecular mass of about 25 kDa.

L8 ANSWER 23 OF 24 MEDLINE

DUPLICATE 9

ACCESSION NUMBER: 90355837 MEDLINE

DOCUMENT NUMBER: 90355837

TITLE: Stable expression of meningococcal class 1 protein in
an antigenically reactive form in outer membranes of
Escherichia coli.

AUTHOR: White D A; Barlow A K; Clarke I N; Heckels J E

CORPORATE SOURCE: Department of Microbiology, University of Southampton
Medical School, Southampton General Hospital, UK..

SOURCE: MOLECULAR MICROBIOLOGY, (1990 May) 4 (5) 769-76.

Journal code: MOM. ISSN: 0950-382X.

PUB. COUNTRY: ENGLAND: United Kingdom

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199011

AB The entire gene encoding the class 1 outer membrane **protein**
of *Neisseria meningitidis* is located on a 2.2kb fragment,
obtained on digestion of chromosomal DNA with XbaI. This
XbaI fragment from strain MC50 (subtype P1-16), which had previously
been cloned in bacteriophage M13, has been transferred to the
plasmid vector pMTL20. The resulting plasmid (pPORA100) was
propagated in *Escherichia coli* (JM109) and cell lysates were
subjected to SDS-PAGE. Western blotting with
anti-class 1 **protein** antibodies revealed constitutive
expression of a **protein** of 41 kD, corresponding to the
class 1 **protein** of the parent meningococcal strain, which
was absent in the *E. coli* control. Fractionation of *E. coli* cells
carrying the recombinant plasmid revealed that the **protein**
was exclusively located in the outer membrane, and N-terminal amino
acid analysis of the expressed **protein** revealed that
normal processing of the signal **peptide** had occurred.
Immuno-gold electron microscopy showed that the protective epitope
recognized by a P1-16 subtype-specific monoclonal antibody was
exposed in an antigenically reactive form on the surface of *E. coli*
cells carrying plasmid pPORA100. In contrast, expression in *E. coli*
of a second plasmid (pPORA104) lacking the coding sequence for the
first 15 amino acids of the signal **peptide** resulted in
accumulation of recombinant class 1 **protein** only in the
cytoplasm of the cells. Thus the presence of the meningococcal
signal sequence ensures expression of this meningococcal porin
protein in an antigenically native conformation in outer
membranes of *E. coli*, while its absence results in expression of a
soluble **protein**. (ABSTRACT TRUNCATED AT 250 WORDS)

L8 ANSWER 24 OF 24 MEDLINE

DUPLICATE 10

ACCESSION NUMBER: 86252506 MEDLINE

DOCUMENT NUMBER: 86252506

TITLE: An unusual *Neisseria* isolated from conjunctival cultures in rural Egypt.

AUTHOR: Mazloum H; Totten P A; Brooks G F; Dawson C R; Falkow S; James J F; Knapp J S; Koomey J M; Lammel C J; Peters D; et al

CONTRACT NUMBER: AI-15642 (NIAID)

AI-21912 (NIAID)

EY-00427 (NEI)

+

SOURCE: JOURNAL OF INFECTIOUS DISEASES, (1986 Aug) 154 (2) 212-24.

Journal code: IH3. ISSN: 0022-1899.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals

ENTRY MONTH: 198610

AB Seven isolates of an unusual *Neisseria* sp. were obtained from eye cultures of children in two rural Egyptian villages. These *Neisseria* utilized only glucose, they exhibited a positive reaction when tested with antisera to crude antigen from *Neisseria meningitidis* and *N. gonorrhoeae*, and they did not react with the fluorescent antibody tests for *N. gonorrhoeae* or with the monoclonal antibodies used to serotype gonococci. The Egyptian isolates had colony morphology more typical of meningococci than gonococci and showed opaque and transparent colony variants. On SDS-PAGE, the major outer-membrane proteins had different patterns than those noted for comparable proteins of meningococci and gonococci; heat-modifiable outer-membrane proteins were present. Four of the six isolates examined had cryptic plasmids of 2.8 megadaltons, which were slightly larger than the cryptic plasmid of *N. gonorrhoeae*. These plasmids were homologous to the gonococcal cryptic plasmid, but had different restriction enzyme fragment patterns. The DNA from the Egyptian isolates, like DNA from *N. meningitidis* but unlike DNA from *N. gonorrhoeae*, could be cut with the restriction enzyme HaeIII. The frequency of transformation into a temperature-sensitive mutant of *N. gonorrhoeae* was 0.2 for the Egyptian isolates and 0.1 for *N. meningitidis*, a frequency that was 5-10-fold lower than that for the *N. gonorrhoeae* control isolates. Whole-cell DNA from the Egyptian isolates showed 68%-73% homology with *N. gonorrhoeae* and 57%-63% with *N. meningitidis*. On the basis of our observations, the Egyptian isolates are distinct from *N. meningitidis* and may represent a variant of *N. gonorrhoeae*. We suggest that the isolates be called *Neisseria gonorrhoeae* ssp. *kochii*.

Searcher : Shears 308-4994

FILE 'CAPLUS' ENTERED AT 09:59:45 ON 22 MAR 2001

L9 6 S L5 AND ((NA OR SODIUM) (W) DODECYL (1W) (PAGE OR (POLYACRYL?
OR POLY ACRYL?) (1W) ELECTROPHOR?)
L10 5 S L9 NOT L6

L10 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:371105 CAPLUS

DOCUMENT NUMBER: 133:218385

TITLE: Sequence variation in the porA gene of a clone
of Neisseria meningitidis during epidemic spread

AUTHOR(S): Jelfs, J.; Munro, R.; Wedege, E.; Caugant, D. A.

CORPORATE SOURCE: WHO Collaborating Centre for Reference and
Research on Meningococci, Department of
Bacteriology, National Institute of Public
Health, Oslo, N-0403, Norway

SOURCE: Clin. Diagn. Lab. Immunol. (2000), 7(3), 390-395
CODEN: CDIMEN; ISSN: 1071-412X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The ET-15 clone within the electrophoretic type (ET)-37 complex of
Neisseria meningitidis was 1st detected in Canada in 1986 and was
since assocd. with outbreaks of meningococcal disease in many parts
of the world. While the majority of the strains of the ET-37
complex are serosubtype P1.5,2, serosubtype detn. of ET-15 strains
may often be incomplete, with either only 1 or none of the 2
variable regions (VRs) of the serosubtype PorA outer membrane
protein reacting with monoclonal antibodies. DNA sequence
anal. of the porA gene from ET-15 strains with 1 or both
unidentified serosubtype determinants was undertaken to identify the
genetic basis of the lack of reaction with the monoclonal
antibodies. 14 Different porA alleles were identified among 38
ET-15 strains from various geog. origins. The sequences
corresponding to subtypes P1.5a,10d, P1.5,2, P1.5,10d, P1.5a,10k,
and P1.5a,10a were identified in 18, 11, 2, 2, and 1 isolate, resp.
Of the remaining 4 strains, which all were nonserosubtypeable, 2 had
a stop codon within the VR1 and the VR2, resp., while in the other 2
the porA gene was interrupted by the insertion element, IS1301. Of
the strains with P1.5,2 sequence, one had a stop codon between the
VR1 and VR2, one had a 4-amino-acid deletion outside the VR2, and
another showed no expression of PorA on sodium
dodecyl sulfate-polyacrylamide gel
electrophoresis. Our results reveal that numerous genetic
events have occurred in the porA gene of the ET-15 clone in the
short time of its epidemic spread. The magnitude of
microevolutionary mechanisms available in meningococci and the
remarkable genetic flexibility of these bacteria need to be
considered in relation to PorA vaccine development.

REFERENCE COUNT: 37

Searcher : Shears 308-4994

REFERENCE(S) :

- (3) Arhin, F; Can J Microbiol 1998, V44, P56
CAPLUS
- (5) Barlow, A; Mol Microbiol 1989, V3, P131
CAPLUS
- (10) Feavers, I; Clin Diagn Lab Immunol 1996,
V3, P444 CAPLUS
- (11) Feavers, I; Mol Microbiol 1992, V6, P489
CAPLUS
- (15) Hammerschmidt, S; EMBO J 1996, V15, P192
CAPLUS

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L10 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:722364 CAPLUS

DOCUMENT NUMBER: 126:27472

TITLE: Two glycosyltransferase genes, lgtF and rfaK,
constitute the lipooligosaccharide ice (inner
core extension) biosynthesis operon of Neisseria
meningitidis

AUTHOR(S): Kahler, Charlene M.; Carlson, Russell W.;
Rahman, M. Mahbubur; Martin, Larry E.; Stephens,
David S.

CORPORATE SOURCE: Deps. Medicine and Microbiol. and Immunology,
Emory Univ. School Medicine, Atlanta, GA, T, USA

SOURCE: J. Bacteriol. (1996), 178(23), 6677-6684

CODEN: JOBAAY; ISSN: 0021-9193

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have characterized an operon required for inner-core biosynthesis of the lipooligosaccharide (LOS) of Neisseria meningitidis. Using Tn916 mutagenesis, the authors recently identified the .alpha.-1,2-N-acetylglucosamine (GlcNAc) transferase gene (rfaK), which when inactivated prevents the addn. of GlcNAc and .alpha. chain to the meningococcal LOS inner core. During the study of rfaK, a second open reading frame (lgtF) of 720 bp was found upstream of rfaK. An amino acid sequence homol. search of the GenBank and EMBL databases revealed that the amino terminus of LgtF has significant homol. with a family of .beta.-glycosyltransferases involved in the biosynthesis of polysaccharides and O antigen of lipopolysaccharides. The chromosomal copy of lgtF was mutagenized with a nonpolar antibiotic resistance cassette to minimize potential polar effects on rfaK. Tricine sodium dodecyl sulfate-polyacrylamide gel electrophoresis and compn. anal. of the LOS from the nonpolar lgtF mutant showed that this strain produced a truncated LOS structure which contained a LOS inner core of GlcNAc1Hep2KDO2 lipid A but without the addn. of lacto-N-neotetraose to HepI or glucose to HepII. These results and the amino acid homol. with .beta.-glycosyltransferases suggest that

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lgtF encodes the UDP-glucose:LOS-.beta.-1,4-glucosyltransferase which attaches the first glucose residue to HepI of LOS. Reverse transcriptase PCR and primer extension anal. indicate that both lgtF and rfaK are cotranscribed as a polycistronic message from a promoter upstream of lgtF. This arrangement suggests that completion of the LOS inner core and the initiation of the .alpha. chain addn. are tightly coregulated in *N. meningitidis*.

L10 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:545679 CAPLUS

DOCUMENT NUMBER: 123:2623

TITLE: Interaction of the *Neisseria gonorrhoeae* PilA protein with the pile promoter involves multiple sites on the

DNA

AUTHOR(S): Arvidson, Cindy Grove; So, Magdalene
CORPORATE SOURCE: Dep. Molec. Microbiol. Immunol., Oregon Health Sci. Univ., Portland, OR, 97201-3098, USA

SOURCE: J. Bacteriol. (1995), 177(9), 2497-504

CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

LANGUAGE: English

AB PilA is the putative DNA-binding component of a two-component system that regulates transcription of the pilin expression locus (pile) of *Neisseria gonorrhoeae*. Here we report the purifn. of the PilA protein and characterization of its DNA-binding activity. PilA was overproduced in *Escherichia coli* with an isopropyl-.beta.-D-thiogalactopyranoside (IPTG)-inducible expression vector. Cell exts. were prepd. by sonication and fractionated by anion-exchange chromatog., followed by dye affinity chromatog. with Cibacron Blue. Proteins were eluted by using a gradient of KCl, and PilA-contg. fractions were identified by immunoblot anal. with a polyclonal anti-PilA antiserum. Purified PilA was judged to be >90% pure, as detd. by Coomassie blue staining and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. PilA purified in this manner was used to develop a gel retardation assay with a 301-bp fragment contg. the pile promoter (Ppile) and upstream sequences as a probe. A fragment of similar size contg. the *E. coli* aroH promoter was used as a neg. control. Competition expts. using a 100- to 1,000-fold excess of unlabeled DNA fragments confirmed the specificity of PilA binding to the pile promoter. To localize the PilA binding site within the 301-bp Ppile fragment, stepwise deletions were generated by PCR and the fragments were examd. in the gel shift assay. The results of these expts. show that there are two regions upstream of Ppile that are required for binding by PilA. Taken together, these data indicate that while PilA binds specifically to the upstream region of the pile gene, this interaction is complex and likely involves multiple regions of

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this DNA sequence.

L10 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:291104 CAPLUS

DOCUMENT NUMBER: 120:291104

TITLE: Genetic diversity of the iron-binding
protein (Fbp) gene of the pathogenic and
commensal **Neisseria**

AUTHOR(S): Genco, Caroline Attardo; Berish, Sally A.; Chen,
Chengyen; Morse, Stephen; Trees, David L.

CORPORATE SOURCE: Dep. Microbiol. Immunol., Morehouse Sch. Med.,
Atlanta, GA, 30310, USA

SOURCE: FEMS Microbiol. Lett. (1994), 116(2), 123-9
CODEN: FMLED7; ISSN: 0378-1097

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The pathogenic **Neisseria** and most commensal
Neisseria species produce an iron-binding **protein**
(Fbp) when grown under iron-limited conditions. In the current
study, the authors confirmed the presence of Fbp, as well as
DNA sequences homologous to the gonococcal fbp, in strains
of *N. gonorrhoeae*, *N. meningitidis*, *N. cinerea*, *N. lactamica*, *N.*
subflava, *N. kochii* and *N. polysaccharea*. The fbp genes from these
strains were amplified by the polymerase chain reaction, digested
with *StuI* or *RsaI*, and the restriction patterns examd. The patterns
for the gonococcal and meningococcal fbp were virtually identical;
however, variations were obsd. in the fbp sequences of the commensal
Neisseria species. *N. flavescens*, *N. mucosa*, *N. sicca*, *N. ovis* and
Branhamella catarrhalis, did not produce Fbp as detected by
sodium dodecyl sulfate-polyacrylamide
gel **electrophoresis** and reactivity with an Fbp specific
monoclonal antibody, nor did they hybridize to an fbp-specific
DNA probe.

L10 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1993:161990 CAPLUS

DOCUMENT NUMBER: 118:161990

TITLE: Analysis of the lsi region involved in
lipooligosaccharide biosynthesis in **Neisseria**
gonorrhoeae

AUTHOR(S): Petricoin, Emanuel F., III; Danaher, Robert J.;
Stein, Daniel C.

CORPORATE SOURCE: Dep. Microbiol., Univ. Maryland, College Park,
MD, 20742, USA

SOURCE: J. Bacteriol. (1991), 173(24), 7896-902
CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The genetic locus (lsi-1) responsible for the transformation of the
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lipooligosaccharide (LOS)-defective *N. gonorrhoeae* mutant FA5100 to LOS expression was studied by deletion mutagenesis and sequence anal. An open reading frame that was preceded by a leader sequence contg. regions with the potential to form hairpin loops was identified. A perfect .sigma.70 promoter consensus was found upstream from this open reading frame. Promoter function was screened for functionality by using lac fusion cassettes and in vitro transcription-translation anal. A frameshift mutation in the lsi-1 gene was constructed by site-directed mutagenesis and introduced into the chromosome of FA19, the LOS-expressing isogenic parent strain of FA5100. The mutant was characterized by Southern blotting, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and Western blotting (immunoblotting) and found to be phenotypically identical to FA5100.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 10:02:13 ON 22 MAR 2001)

L11 30 S L9
L12 24 S L11 NOT L7
L13 8 DUP REM L12 (16 DUPLICATES REMOVED)

L13 ANSWER 1 OF 8 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 2001035739 EMBASE

TITLE: Analysis of lipooligosaccharide biosynthesis in the *Neisseriaceae*.

AUTHOR: Arking D.; Tong Y.; Stein D.C.

CORPORATE SOURCE: D.C. Stein, Dept. of Cell Biology/Molec. Genet.,
University of Maryland, College Park, MD 20742,
United States. DS64@UMAIL.UMD.EDU

SOURCE: Journal of Bacteriology, (2001) 183/3 (934-941).
Refs: 51
ISSN: 0021-9193 CODEN: JOBAAY

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Neisserial lipooligosaccharide (LOS) contains three oligosaccharide chains, termed the .alpha., .beta., and .gamma. chains. We used Southern hybridization experiments on DNA isolated from various *Neisseria* spp. to determine if strains considered to be nonpathogenic possessed DNA sequences homologous with genes involved in the biosynthesis of these oligosaccharide chains. The presence or absence of specific genes was compared to the LOS profiles expressed by each strain, as characterized by their mobilities on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and their reactivities with various LOS-specific monoclonal antibodies. A

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great deal of heterogeneity was seen with respect to the presence of genes encoding glycosyltransferases in *Neisseria*. All pathogenic species were found to possess DNA sequences homologous with the lgt gene cluster, a group of genes needed for the synthesis of the .alpha. chain. Some of these genes were also found to be present in strains considered to be nonpathogenic, such as *Neisseria lactamica*, *N. subflava*, and *N. sicca*. Some nonpathogenic *Neisseria* spp. were able to express high-molecular-mass LOS structures, even though they lacked the DNA sequences homologous with rfaF, a gene whose product must act before gonococcal and meningococcal LOS can be elongated. Using a PCR amplification strategy, in combination with DNA sequencing, we demonstrated that *N. subflava* 44 possessed lgtA, lgtB, and lgtE genes. The predicted amino acid sequence encoded by each of these genes suggested that they encoded functional proteins; however, structural analysis of LOS isolated from this strain indicated that the bulk of its LOS was not modified by these gene products. This suggests the existence of an additional regulatory mechanism that is responsible for the limited expression of these genes in this strain.

L13 ANSWER 2 OF 8 MEDLINE DUPLICATE 1
 ACCESSION NUMBER: 2000261945 MEDLINE
 DOCUMENT NUMBER: 20261945
 TITLE: Sequence variation in the porA gene of a clone of *Neisseria meningitidis* during epidemic spread.
 AUTHOR: Jelfs J; Munro R; Wedege E; Caugant D A
 CORPORATE SOURCE: WHO Collaborating Centre for Reference and Research on Meningococci, Department of Bacteriology, National Institute of Public Health, N-0403 Oslo, Norway.
 SOURCE: CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (2000 May) 7 (3) 390-5.
 Journal code: CB7. ISSN: 1071-412X.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200009
 ENTRY WEEK: 20000901
 AB The ET-15 clone within the electrophoretic type (ET)-37 complex of *Neisseria meningitidis* was first detected in Canada in 1986 and has since been associated with outbreaks of meningococcal disease in many parts of the world. While the majority of the strains of the ET-37 complex are serosubtype P1.5,2, serosubtype determination of ET-15 strains may often be incomplete, with either only one or none of the two variable regions (VRs) of the serosubtype PorA outer membrane protein reacting with monoclonal antibodies. DNA sequence analysis of the porA gene from ET-15 strains with one or both unidentified serosubtype
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determinants was undertaken to identify the genetic basis of the lack of reaction with the monoclonal antibodies. Fourteen different *porA* alleles were identified among 38 ET-15 strains from various geographic origins. The sequences corresponding to subtypes P1.5a,10d, P1.5,2, P1.5,10d, P1.5a,10k, and P1.5a,10a were identified in 18, 11, 2, 2, and 1 isolate, respectively. Of the remaining four strains, which all were nonserosubtypeable, two had a stop codon within the VR1 and the VR2, respectively, while in the other two the *porA* gene was interrupted by the insertion element, IS1301. Of the strains with P1.5,2 sequence, one had a stop codon between the VR1 and VR2, one had a four-amino-acid deletion outside the VR2, and another showed no expression of PorA on **sodium dodecyl sulfate-polyacrylamide gel electrophoresis**. Our results reveal that numerous genetic events have occurred in the *porA* gene of the ET-15 clone in the short time of its epidemic spread. The magnitude of microevolutionary mechanisms available in meningococci and the remarkable genetic flexibility of these bacteria need to be considered in relation to PorA vaccine development.

L13 ANSWER 3 OF 8 MEDLINE DUPLICATE 2
 ACCESSION NUMBER: 97149287 MEDLINE
 DOCUMENT NUMBER: 97149287
 TITLE: Cloning, sequencing and expression of an *Eikenella corrodens* gene encoding a component protein of the lectin-like adhesin complex.
 AUTHOR: Yumoto H; Azakami H; Nakae H; Matsuo T; Ebisu S
 CORPORATE SOURCE: Department of Conservative Dentistry, Tokushima University School of Dentistry, Japan..
 yumoto@dent.dent.tokushima-u.ac.jp
 SOURCE: GENE, (1996 Dec 12) 183 (1-2) 115-21.
 Journal code: FOP. ISSN: 0378-1119.
 PUB. COUNTRY: Netherlands
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-D70829
 ENTRY MONTH: 199704
 ENTRY WEEK: 19970403
 AB A lectin-like substance (LS), that was isolated from *Eikenella corrodens* (Ec) 1073, migrated as **proteins** of about 300 and 45 kDa upon **sodium dodecyl sulfate-polyacrylamide gel electrophoresis** under reducing conditions. In this study, we cloned the gene encoding the 45-kDa **protein** and predicted its structure and function. Based on the N-terminal 23-amino acid (aa) sequence of this **protein**, we cloned the region for its N-terminus. We cloned the entire gene by means of gene walking using polymerase chain reaction and Southern hybridization. The **nucleotide** sequences of cloned
 Searcher : Shears 308-4994

fragments revealed an open reading frame encoding a **polypeptide** of 330 aa (M(r), 35748). This ORF displayed high homology to those of porins of *Neisseria* species. Using the T7-expression system, the 45-kDa **protein** was produced in *E. coli*. Our results suggested that the 45-kDa **protein** of Ec 1073 is a component of the EcLS complex, and that it is the major outer membrane **protein**.

L13 ANSWER 4 OF 8 MEDLINE DUPLICATE 3
 ACCESSION NUMBER: 95247686 MEDLINE
 DOCUMENT NUMBER: 95247686
 TITLE: Interaction of the *Neisseria gonorrhoeae* Pila **protein** with the pile promoter involves multiple sites on the DNA.
 AUTHOR: Arvidson C G; So M
 CORPORATE SOURCE: Department of Molecular Microbiology and Immunology, Oregon Health Sciences University, Portland 97201-3098, USA..
 CONTRACT NUMBER: AI20845 (NIAID)
 AI07354 (NIAID)
 SOURCE: JOURNAL OF BACTERIOLOGY, (1995 May) 177 (9) 2497-504.
 Journal code: HH3. ISSN: 0021-9193.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199508

AB Pila is the putative DNA-binding component of a two-component system that regulates transcription of the pilin expression locus (pile) of *Neisseria gonorrhoeae*. Here we report the purification of the Pila **protein** and characterization of its DNA-binding activity. Pila was overproduced in *Escherichia coli* with an isopropyl-beta-D-thiogalactopyranoside (IPTG)-inducible expression vector. Cell extracts were prepared by sonication and fractionated by anion-exchange chromatography, followed by dye affinity chromatography with Cibacron Blue. **Proteins** were eluted by using a gradient of KCl, and Pila-containing fractions were identified by immunoblot analysis with a polyclonal anti-Pila antiserum. Purified Pila was judged to be > 90% pure, as determined by Coomassie blue staining and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Pila purified in this manner was used to develop a gel retardation assay with a 301-bp fragment containing the pile promoter (Ppile) and upstream sequences as a probe. A fragment of similar size containing the *E. coli* aroH promoter was used as a negative control. Competition experiments using a 100- to 1,000-fold excess of unlabelled DNA fragments confirmed the specificity of Pila binding to the pile promoter. To localize the Pila binding site

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within the 301-bp PpIle fragment, stepwise deletions were generated by PCR and the fragments were examined in the gel shift assay. The results of these experiments show that there are two regions upstream of PpIle that are required for binding by Pila. (ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 5 OF 8 MEDLINE DUPLICATE 4
 ACCESSION NUMBER: 94200567 MEDLINE
 DOCUMENT NUMBER: 94200567
 TITLE: Genetic diversity of the iron-binding protein
 (Fbp) gene of the pathogenic and commensal
Neisseria.
 AUTHOR: Genco C A; Berish S A; Chen C Y; Morse S; Trees D L
 CORPORATE SOURCE: Department of Microbiology and Immunology, Morehouse
 School of Medicine, Atlanta, Georgia 30310..
 CONTRACT NUMBER: AI30797 (NIAID)
 SOURCE: FEMS MICROBIOLOGY LETTERS, (1994 Feb 15) 116 (2)
 123-9.
 Journal code: FML. ISSN: 0378-1097.
 PUB. COUNTRY: Netherlands
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199407
 AB The pathogenic **Neisseria** and most commensal
Neisseria species produce an iron-binding protein
 (Fbp) when grown under iron-limited conditions. In the current
 study, we confirmed the presence of Fbp, as well as DNA
 sequences homologous to the gonococcal fbp, in strains of N.
 gonorrhoeae, N. meningitidis, N. cinerea, N. lactamica, N. subflava,
 N. kochii and N. polysaccharea. The fbp genes from these strains
 were amplified by the polymerase chain reaction, digested with StuI
 or RsaI, and the restriction patterns examined. The patterns for the
 gonococcal and meningococcal fbp were virtually identical; however,
 variations were observed in the fbp sequences of the commensal
Neisseria species. N. flavescens, N. mucosa, N. sicca, N.
 ovis and Branhamella catarrhalis, did not produce Fbp as detected by
 sodium dodecyl sulfate-polyacrylamide
 gel electrophoresis and reactivity with an Fbp specific
 monoclonal antibody, nor did they hybridize to an fbp-specific
 DNA probe.

L13 ANSWER 6 OF 8 MEDLINE DUPLICATE 5
 ACCESSION NUMBER: 94059745 MEDLINE
 DOCUMENT NUMBER: 94059745
 TITLE: **Neisseria weaveri** sp. nov. (formerly CDC group M-5),
 from dog bite wounds of humans.
 AUTHOR: Holmes B; Costas M; On S L; Vandamme P; Falsen E;
 Kersters K
 Searcher : Shears 308-4994

CORPORATE SOURCE: Epidemiological Identification and Typing Laboratory,
Central Public Health Laboratory, London, United
Kingdom..

SOURCE: INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY,
(1993 Oct) 43 (4) 687-93.
Journal code: AWO. ISSN: 0020-7713.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199403

AB The taxonomic relationships of strains belonging to Centers for
Disease Control group M-5 were examined. Previous studies of rRNA
cistron similarities placed this organism on the Neisseriaceae rRNA
branch of rRNA superfamily III; the closest neighbors included the
genus *Neisseria* and groups EF-4a and EF-4b. The group M-5
strains were characterized by a range of phenotypic tests, and their
G + C contents and DNA-DNA relatedness levels
were determined. In addition, a numerical taxonomic analysis of the
whole-cell protein patterns (obtained by sodium
dodecyl sulfate-polyacrylamide gel
electrophoresis) of group M-5 and related taxa was
performed. The strains studied included 45 group M-5 strains, the
type strains of six *Neisseria* species or subspecies, three
group EF-4a reference strains, and three group EF-4b reference
strains plus the type strain of the phenotypically similar organism
Oligella urethralis. Our results showed that the group M-5 strains
were members of a homogeneous taxon distinct from phylogenetically
closely related taxa. The genomic divergence as revealed by levels
of rRNA cistron similarity and phenotypic characteristics indicate
that group M-5 can be considered a new species of the genus
Neisseria. We therefore propose the new species
Neisseria weaveri, with NCTC 12742 (= CCUG 4007 = ISL775/91
= LMG 5135) as the type strain. *N. weaveri* strains are strictly
aerobic, gram-negative, nonmotile, rod-shaped organisms which are
catalase and oxidase positive, nonsaccharolytic, and able to grow on
MacConkey agar and do not reduce nitrate but generally reduce
nitrite. (ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 7 OF 8 MEDLINE DUPLICATE 6

ACCESSION NUMBER: 93084746 MEDLINE

DOCUMENT NUMBER: 93084746

TITLE: Identification of meningococcal serosubtypes by
polymerase chain reaction.

AUTHOR: Maiden M C; Bygraves J A; McCarvil J; Feavers I M

CORPORATE SOURCE: National Institute for Biological Standards and
Control, Potters Bar, Hertfordshire, United Kingdom..

SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (1992 Nov) 30 (11)
2835-41.

Searcher : Shears 308-4994

09/7388090

JOURNAL CODE: HSH. ISSN: 0095-1137.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303

AB The polymerase chain reaction was used as the basis of a novel typing method for *Neisseria meningitidis*. Southern hybridization experiments demonstrated that it was possible to identify genes encoding different serological variants of the meningococcal class 1 outer membrane **protein** by probing with polymerase chain reaction products corresponding to known epitopes. A set of 14 defined variable regions was prepared in bacteriophage M13mp19 by the cloning of polymerase chain reaction products. The phage were dot blotted onto membrane filters, which were used as targets for hybridization of radiolabeled amplified class 1 outer membrane **protein** genes. Thus, the presence of many different subtype-specific epitopes could be investigated in one experiment. This technique was evaluated with a set of serological reference strains, mainly of serogroup B organisms, and provided an alternative, rapid, and comprehensive typing system that was capable of distinguishing known serosubtypes and also of defining currently untypeable strains independently of **sodium dodecyl sulfate-polyacrylamide** gel **electrophoresis** or serological analysis. An additional advantage of this technique was that in the case of an unknown serosubtype (i.e., one that did not hybridize with any of the known samples), the **DNA** amplified from the original sample could be used to determine the **nucleotide** sequence of the novel serosubtype and to clone the corresponding variable region into bacteriophage M13. It may be possible to develop this procedure for the diagnostic detection and typing of meningococci directly from clinical samples even when culture is not possible because of antibiotic treatment of an acute case.

L13 ANSWER 8 OF 8 MEDLINE
ACCESSION NUMBER: 86149857 MEDLINE
DOCUMENT NUMBER: 86149857
TITLE: **Polypeptides** encoded by cryptic plasmids from *Neisseria gonorrhoeae*.
AUTHOR: Aalen R B; Gundersen W B
SOURCE: PLASMID, (1985 Nov) 14 (3) 209-16.
JOURNAL CODE: P8P. ISSN: 0147-619X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198606
AB Almost all clinical isolates of *Neisseria gonorrhoeae*
Searcher : Shears 308-4994

harbor a small, phenotypically cryptic plasmid of approximately 4.1 kb. In this study several **polypeptides** encoded by two variants of such plasmids, one (pSB01C) having a deletion of approximately 50 bp as compared to the other (p31788C), have been identified, and the position of the genes for two of the **proteins** determined. The cryptic plasmids were cloned into the HindIII site of the vectors pBR322 and pACYC184. The resulting recombinant plasmids were transformed into the Escherichia coli minicell producing strain DS410 (minA, minB) and the plasmid-encoded **proteins** analyzed by **sodium dodecyl sulfate-polyacrylamide gel electrophoresis**. The pSB01C derivatives express two distinct **proteins** of 22 and 16 kDa and p31788C two other **proteins** of 24 and 18.5 kDa. Additionally, both plasmids express common **proteins** of 32.5, 9, and 7.5 kDa. The genes coding for the 24- and the 7.5 kDa **proteins** have been mapped by restriction enzyme analysis of Tn5 insertions suppressing the expression. The additional 50 bp in p31788C are localized to the coding region of the 24-kDa **protein**, and the 22-kDa **protein** of pSB01C is possibly a shortened form of the former due to the lacking 50 bp.

FILE 'HOME' ENTERED AT 10:03:57 ON 22 MAR 2001

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FILE 'CAPLUS' ENTERED AT 10:20:57 ON 22 MAR 2001

L4 1688 SEA FILE=CAPLUS ABB=ON PLU=ON NEISSERIA(S) (POLYPEPTIDE
OR PEPTIDE OR PROTEIN OR POLYPROTEIN)
L5 609 SEA FILE=CAPLUS ABB=ON PLU=ON L4 AND (DNA OR DEOXYRIBON
CULEIC OR DEOXY RIBONUCLEIC OR NUCLEOTIDE)
L14 51368 SEA FILE=CAPLUS ABB=ON PLU=ON (SDS OR (NA OR SODIUM) (W)
DODECYL) (1W) (PAGE OR (POLYACRYL? OR POLY ACRYL?) (3W)ELECT
ROPHOR?)
L15 24 SEA FILE=CAPLUS ABB=ON PLU=ON L5 AND L14

L16 0 S L15 NOT (L6 OR L9)

FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT,
TOXLINE, PHIC, PHIN' ENTERED AT 10:31:15 ON 22 MAR 2001

L17 72 S L15
L18 0 S L17 NOT (L7 OR L12)

=> fil hom

FILE 'HOME' ENTERED AT 10:33:35 ON 22 MAR 2001